BAFFIN ISLAND EXPERIMENTAL OIL SPILL AND DISPERSANT STUDIES. HYDROCARBON BIOACCUMULATION AND HISTOPATHOLOGICAL AND BIOCHEMICAL RESPONSES IN MARINE BIVALVE MOLLUSCS

by

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SUMMARY

Infaunal bivalve molluscs from four bays at the BLOS experimental oil spill site became contaminated with petroleum hydrocarbons. Bay 7 was considered a reference bay (though it received some oil), Bays 9 and 10 received dispersed oil, and Bay 11 received oil alone. A Lagomedio crude oil and the dispersant, Corexit 9527, were usedin these field experiments. Mya truncata and Serripes groenlandicus, which are filter-feeders, rapidly accumulated dispersed oil in Bays 7, 9 and 10 immediately after the spill, but released much of the hydrocarbons by the second post-spill sampling about two weeks after the spill. The deposit feeders, Macoma calcarea, Astarte borealis, and Nuculana minuta, accumulated more oil than did the filter-feeders (presumably from the sediments) and retained them longer in Bays 9 and 10. In Bay 11, all five species accumulated very little oil immediately after the spill but became heavily contaminated within about two weeks. Bay 7 received about 50-100 ppb dispersed oil in the first few days after the dispersed oil spill. This was about 1,000-fold less than the amount in the water of Bay 9. Nevertheless, the molluscs, especially Serripes, from Bay 7 became moderately heavily contaminated with oil.

Based on chemical data, both Mya and Serripes depurated oil during the two-week post-spill period, in part through an in vivo biodegradation presumably by microbial activity in the guts of the animals. However, Serripes preferentially retained the high molecular weight saturated hydrocarbon assemblage as well as the higher alkylated naphthalene, phenanthrene and dibenzothiophene compounds, whereas Mya depurated all hydrocarbon components although the water-soluble alkyl benzenes and naphthalenes were depurated somewhat faster. The filter-feeders depurated oil even though the sediments in which they resided still contained oil. However, the deposit feeders continued to accumulate oil from the sediments, at least for the two weeks after the spills.

Specimens of Mya truncata and Macoma calcarea for histopathologic examination were collected immediately before, immediately after, and one year after the experimental oil spills. Immediately after the spill, there was an increased incidence of gill and digestive tract necrosis in Mya from the bays receiving dispersed oil (Bays 7, 9 and 10). This was accompanied by an increase in the number of mucus cells in the digestive tract epitheliums. After one year, a few clams had granulocytomas throughout

the tissues. Three clams from Bay 11 (receiving oil alone) collected one year after the spill had invasive neoplasias (probably cancer). One clam from Bay 7 immediately after the spill had a similar lesion.

There were few lesions in Macoma from Bays 7 and 9 immediately after or one year after the spill. One year after the spill, animals from Bay 11 had a high incidence of vacuolization of the digestive tubule epitheliums The incidence of parasitism and hemocytic infiltration also was higher in Macoma from Bay 11 than from the other bays. One specimen had a blood neoplasm.

Clams Mya truncata were collected immediately before, immediately after, and about two weeks after the simulated oil spills for biochemical analysis. Concentrations in the clam tissues of glucose, glycogen, trehalose, total lipid and free amino acids were measured. Concentrations and ratios of free amino acids in adductor muscle were the most useful indices of pollutant stress.

The results of the biochemical analyses indicate that Mya from the four bays were not severely stressed by either dispersed oil or oil alone. Immediately after the spill, clams from the two major dispersed oil bays, and particularly Bay 10, appeared to be more severely stressed than clams from Bay 11 (using clams from Bay 7 as reference). After two weeks, clams from the dispersed oil bays were nearly normal, while those from the bay receiving oil alone appeared stressed. These results seem to corroborate results from analytical chemistry and histopathology, that the acute effects of dispersed oil are greater than those of undispersed oil, but effects of undispersed oil on infaunal molluscs develop more slowly and persist longer than those from dispersed oil.

FINAL REPORT

on

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1. INTRODUCTION

More than 10,000 tons of chemical dispersant were used to clean the coast of Cornwall, England of Kuwait crude oil following the Torrey Canyon oil spill in 1967. It is now generally agreed that the dispersant caused more damage to the intertidal fauna and flora than did the oil itself (Southward and Southward, 1978). The most frequently used dispersant during the Torrey Canyon cleanup contained 12% nonionic surfactant and 3% stabilizer in a high aromatic solvent (kerosene extract). This mixture was highly toxic to nearly all forms of marine life. Because of the disastrous consequences of dispersant use in this and a few other spills, use of chemical dispersants for oil spill cleanup fell into disfavor. Relatively little dispersant was used after the Amoco Cadiz spill and none was used for shoreline cleaning.

Since the Torrey Canyon incident, considerable progress has been made in developing dispersants that have a very low toxicity to marine organisms. Since dispersal may be the method of choice in many cases for treating spilled oil, there is an urgent need for information about the toxicity and environmental impact of oil that has been dispersed with the new generation of "low-toxicity" dispersants (Sprague et al., 1982). The controlled experimental oil spill-dispersant study - The Baffin Island Oil Spill (BIOS) Project - being conducted by the Canadian Environmental Protection Service offers a unique opportunist y to assess the biological effects of dispersed oil in a field situation. The primary objective of the BIOS Project was to determine if the use of dispersants in the Arctic nearshore will reduce or increase the environmental effects of spilled oil (Blackall, 1980).

1.1 Objectives of the Research Program

The primary objective of this research program was to assess and compare sublethal biological effects of chemically dispersed and non-dispersed spilled oil on benthic infaunal bivalve molluscs from the Arctic. The research project has three components: accumulation by three species of molluscs (Mya truncata, Serripes groenlandicus, and Astarte borealis) of hydrocarbons from dispersed and non-dispersed spilled crude petroleum; sublethal biochemical responses of Mya truncata to dispersed and non-dispersed spilled crude petroleum; histopathology of Mya truncata and Macoma calcarea up to one year after the simulated oil spills. The program was designed to determine if chemically dispersed oil is more or less bioavailable than undispersed oil to benthic infaunal bivalve molluscs, and whether dispersed oil is more harmful than undispersed oil to these animals.

1.2 Background

1.2.1 Hydrocarbon Accumulation. Marine animals readily accumulate petroleum hydrocarbons in their tissues from dispersion or solution in seawater and to a lesser extent from petroleum-contaminated sediments and food (Neff. Anderson, Cox, Laughlin, Rossi and Tatem 1976 ; Neff, Cox, Dixit and Anderson, 1976; Boehm and Quinn, 1977; Lee, 1977; Neff, 1979; Neff and Anderson, 1981; Boehm, Barak, Fiest and Elskus 1982). Bivalve molluscs, apparently because

they have little or no ability to metabolize aromatic hydrocarbons to water-soluble and easily excreted metabolizes (Vandermeulen and Penrose. 1978; Lee, 1981), tend to accumulate petroleum hydrocarbons to higher concentrations and retain them longer than do other phyla of marine organisms (Neff, Cox, Dixit and Anderson 1976; Boehm and Quinn, 1977; Neff and Anderson, 1981; Elmgren et al., 1983). Dispersants favor the formation of micro oil droplets in the water column. The oil droplets are of a size that might be readily filtered from the water and ingested during normal filter feeding activity of bivalve molluscs. Thus, the use of dispersant could increase the bioavailabilit y of petroleum hydrocarbons and, of particular importance, the poorly soluble medium molecular weight polycyclic aromatic hydrocarbons and heterocyclics (azaarenes, dibenzothiophenes, etc.) to bivalve molluscs.

1.2.2 Histopathology. Petroleum hydrocarbons, and particularly the more toxic aromatics and heterocyclics, accumulated by marine animals interact with cells and tissues to produce a variety of lesions. Aromatic hydrocarbons bind to the surface of cell membranes and interfere with cell membrane-mediated biological processes (Roubal, 1974; Roubal and Collier, 1975). Many hydrocarbons are irritants and cause localized inflammatory responses. In oysters Crassostrea gigas from the Amoco Cadiz oil spill site, the most common histopathology was leucocytosis (an inflammatory response) in mantle and gill tissues @Jeff and Haensly, 1982). Cockles, Cerastoderma edule, and mussels, Mytilus edulis, transplanted to a bay that was heavily contaminated with oil from the Amoco Cadiz spill, developed accumulations of lipid droplets and lysosomal granules in the digestive diverticula (Wolfe et al., 1981). Stainken (1976) reported generalized leucocytosis in the mantle of soft-shell clams Mya arenaria exposed in the laboratory to oil. He also observed glycogen depletion and cellular vacuolization in several tissues of exposed clams. A wide variety of other histopathological lesions have been reported in invertebrates and fish exposed to petroleum in the laboratory or field (Malins, 1982).

Crude petroleum and heavy refined oils (e.g., bunker C residual oil) contain known carcinogens including benzo(a)pyrene, dimethylbenz(a)anthracene, and methyl chrysene (Neff, 1979). There are several reports in the literature of increased incidence of apparently cancerous tumors in populations of bivalve molluscs from oil spill sites (Barry and Yevich, 1975; Gardner et al., 1975; Farley, 1977; Yevich and Barszcz, 1977; Brown et al., 1979; Mix, 1982). However, in no case has it been unequivocality demonstrated that oil was the immediate cause of the cancerous lesions.

Immunosuppression and the resulting increased susceptibility to disease, including parasitism, has been observed in molluscs and other marine animals exposed to oil spills (Hodgins et al., 1977; Sindermann, 1982). Since some hyperplastic or neoplastic (cancer-like) lesions in molluscs are known or suspected of being caused by viruses, bacteria, or fungi (Couch and Winstead, 1979), similar cancer-like lesions in bivalves from oil spill sites may result from petroleum-mediated infection with pathogenic organisms.

1.2.3 Biochemistry/Physiology. Several physiological or biochemical measures of metabolic energy partitioning and nutritional status may be sensitive indices of sublethal pollutant stress in marine invertebrates. This conclusion is based on the hypothesis, supported by substantial experimental data, that a majority of pollutants at environmentally realistic concentrations, which are usually well below concentrations that are acutely lethal, act as loading stressors. Chronic exposure of the animal to these sublethal pollutant concentrations leads to an increase in the metabolic cost of basic biological maintenance and homeostatic functions. Less energy is available for growth and reproductive processes, and nutrient reserves are depleted. Recent reviews supporting this hypothesis include those of Rosenthal and Alderdice (1976) and Bayne et al. (1979; 1982).

Typical responses of bivalve molluscs to chronic exposure to sublethal concentrations of petroleum include alterations in **respiration rate or ratio of ox ygen** consumed to nitrogen excreted (Capuzzo, 1981; Widdows et al., 1982), reduction in nutrient assimilation and scope for growth (Dow, 1975; Gilf illan et al., 1976; Gilf illan and Vandermeulen, 1978; Keck et al., 1978; Stekoll et al., 1980; Bayne et al., 1982; Mahoney and Noyes, 1982), reduced growth rate (Anderson et al., 1983), depletion of gl ycogen reserves (Stainken, 1976), changes **in tissue free amino acid concentrations and ratios (Jeff ries, 1972; Roesijadi and Anderson, 1979; Augenfeld et al., 1980), and decrease** in condition index (Roesijadi and Anderson, 1979; Augenfeld et al., 1980). All these responses are indicative of a pollutant-mediated increase in metabolic load (loading stress) on the animals.

In oysters from the Amoco Cadiz oil spill site, we have observed statistically significant long-term (more than two years) changes in tissue free amino acid ratios, blood glucose concentration, and reserves of glycogen and ascorbic acid (Nef f and Haensley, 1982).

2. HYDROCARBON BIOACCUMULATION

2.1 Materials and Methods

Specimens of Mya truncata, Serripes groenlandicus, and Astarte borealis were collected, when available in sufficient numbers, from the 3-meter and 7-meter transects in all four bays (Figure 2.1) at three sampling times, immediately pre-spill, immediately post-spill, and approximately two weeks after the experimental spills. Animals were wrapped in aluminum foil and frozen for air shipment to the laboratory.

Aromatic hydrocarbons and sulfur heterocyclics in tissues were analyzed by gas chromatography/mass spectrometry/data systems (GC/MS/DS). In order to investigate the polycyclic aromatic nitrogen heterocyclic (PANH) composition and content of the tissue, sample extracts from molluscs taken along the two depth strata were pooled and analyzed by GC/MS for PANH.

Very briefly, the analytical methods used were identical to those of Boehm, Barak, Fiest and Elskus (1982], a modification of the Warner (1976) alkaline digestion-extraction procedure. After fractionating the extract on an alumina-silica acid column, the saturated and aromatic hydrocarbons were analyzed by capillary CC and computer-assisted GC/MS (GD/MS/DS) . GS/MS/DS analyses focused on the two- to five-ringed aromatic compounds. PANH analyses involved the GC/MS analysis of an aqueous acid extract of the total extractable (solvent) lipids, which had been neutralized and back extracted with solvent to recover the basic PANH compounds.

2.2 Results

Results from Boehm (1982) of analyses of total saturate and aromatic hydrocarbons in five species of bivalves, including the three species treated in detail in this report, are sum marized in Table 2.1. The three filter-feeders, Mya truncata, Serripes groenlandicus, and Astarte borealis from the bays receiving dispersed oil (Bays 7, 9 and 10) rapidly accumulated petroleum hydrocarbons to high levels within a few days of the spills. In Bay 11 which received undispersed oil, these species accumulated petroleum hydrocarbons more slowly. Animals from the three bays receiving dispersed oil, released

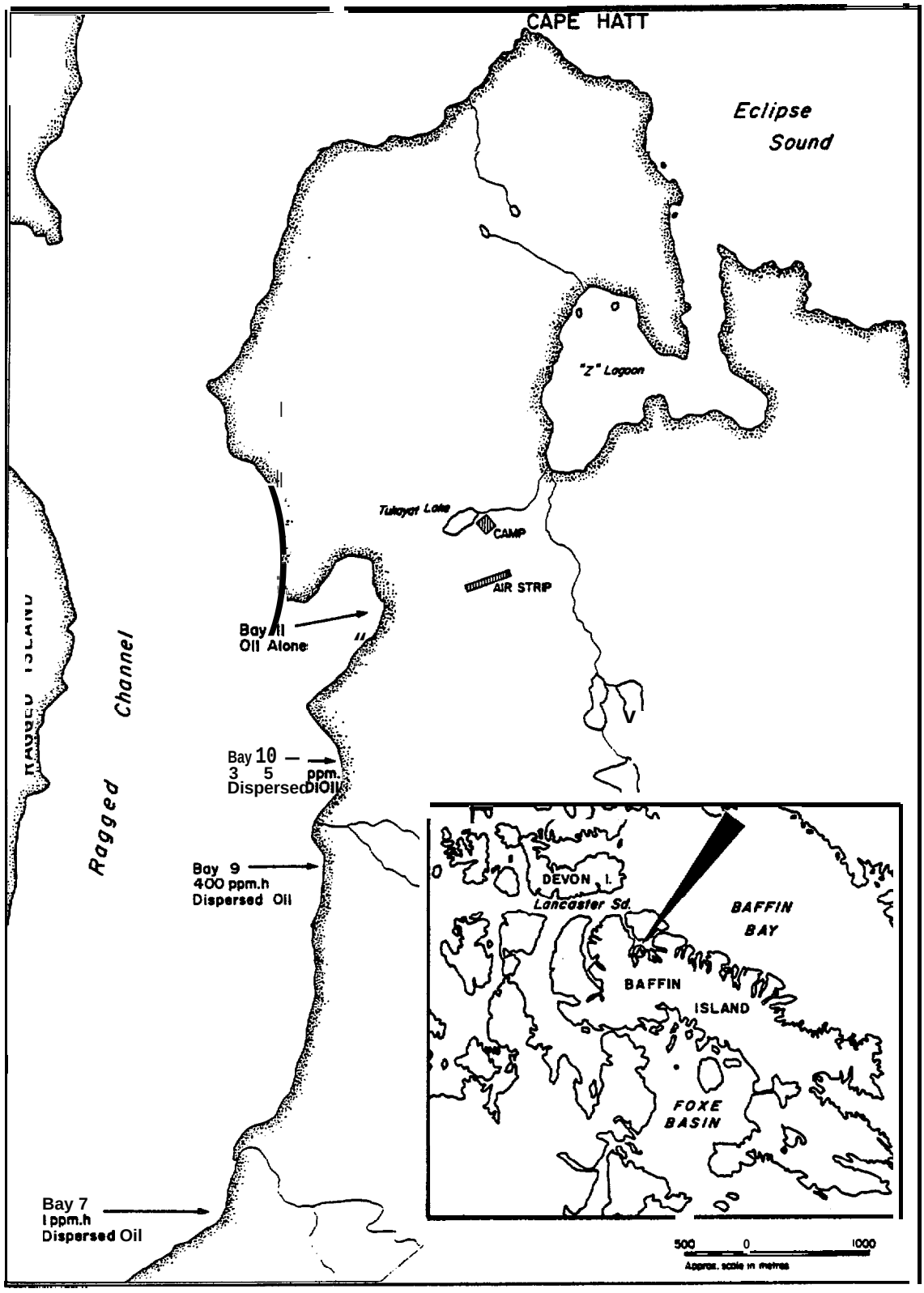

Figure 2.1. BIOS site at Cape Hatt, Baffin Island, showing the locations of study bays and oil treatments applied in August, 1981. Dispersed oil concentrations are maximum estimated exposures in ppm x hours (From Cross and Thompson, 1982).

Table 2.1. **Summary of oil concentrations in mollusc tissues by bay (in µg/g dry weight). (from Boehm 1982)**

SPECIES	STRATUM	BAY 9 (DISPERSED OIL) PRE-SPILL	BAY 9 (DISPERSED OIL) FIRST POST-SPILL	BAY 9 (DISPERSED OIL) SECOND POST-SPILL	BAY 10 (DISPERSED OIL) PRE-SPILL	BAY 10 (DISPERSED OIL) FIRST POST-SPILL	BAY 10 (DISPERSED OIL) SECOND POST-SPILL
Mya truncata	7m	0.35 (.22, .49)	121 (51, 290)	114 (90, 140)	0.57 (.42, .74)	277 (180, 420)	157 (110, 230)
	3m	0.40 (.25, .56)	215 (130, 350)	135 (120, 150)	0.78 (.55, 1.0)	368 (290, 460)	131 (96, 178)
Serripes groenlandicus	7m		186 (110, 330)	97 (59, 160)		329 (240, 460)	141 (110, 180)
	3m airlift			160 (120, 210)		698 (500, 970)	177
	7m	0.68 (.02, 1.9)	482 (340, 680)	116 (69, 190)	1.4 (.40, 3.0)	278 (220, 350)	149 (130, 170)
Macoma calcarea	7m	0.73 (.33, 1.2)	75 (36, 150)	836 (61O, 1140)	2.1 (1.0, 3.6)	406 (241, 680)	440 (250, 760)
	3m						
Astarte borealis	7m	0.81 (0.41, 1.3)	463 (270, 800)	171 (88, 330)	1.4	441.5	336.7
	3m						
Nuculana minuta	7m	1.3	33.0	615.6	1.4	441.5	336.7
	3m						

Table 2.1. (Continued)

SPECIES	STRATUM	BAY 7 (REFERENCE) PRE-SPILL	FIRST POST-SPILL	SECOND POST-SPILL	BAY 11 (OIL ALONE) PRE-SPILL	FIRST POST-SPILL	SECOND POST-SPILL
Mya truncata	7m	0.34 (.2 1, 4.8)	114 (64, 21O)	47 (31, 70)	0.43 (*33, .53)	2.0 (1.2, 3.0	93 (73, 120)
	3m						
Serripes groenlandicus	7m						
	3m airlift						
	7m	1.2 (1.2, 1.3)	517 (360, 750)	73 (31, 170)	1.6	6.0 (.19, 41)	394 (200, 780)
Macoma calcarea	7m	1.0 (.88, 1.2)	82 (60, 112)	85 (39, 190)	2.5 (.05, 10)	24 (14, 42)	246 (76, 790)
	3m						
Astarte borealis	7m	2.2 (.38, 6.4)	51 (12, 210)	& 140)	0.47 (.31, .92)	2.7 (2.2, 394)	140 (50, 390)
	3m						
Nuculana minuta	7m	1.2	41.2	87.3	1.1	11.3	428.9
	3m						

[a]Geometric mean (lower 95% confidence limit, upper 95% confidence limit).

some of the oil during the period between the first and second post-spill sampling (about 2 weeks). A different pattern of hydrocarbon bioaccumulation was evident in the two deposit-feeding bivalves, Macoma calcarea and Nuculana minuta. In these species, uptake of petroleum hydrocarbons in all four bays was more gradual and maximum body burdens were reached in the second post-spill samples.

Although Bay 7 was considered a reference bay, 50-100 ppb dispersed and soluble petroleum hydrocarbons were measured in the water column of the bay after the dispersed oil spill. The benthic bivalves from this bay, in particular Serripes groenlandicus and Mya truncata, became contaminated with petroleum hydrocarbons immediately after the spill.

2.2.1. Mya truncata. The analytical results from 20 samples of Mya truncata are summarized in Figures 2.2-2.15. Results correspond to one GC/MS/DS analysis of a pooled extract of five stations along a depth stratum. For example, the l-day post-spill sample from Bay 9 (7m) represents a result of a pooling of five samples (1 sample . 10 animals) along the 7-meter depth stratum in this bay. Pre-spill, l-day post-spill, and 2-week post-spill analyses are presented for each bay. A set of samples from the inshore (3-meter) transect was analyzed from Bays 9 and 10. In addition to the pooled 5-station sample, analyses were conducted on animals from two individual stations in Bay 9. Total petroleum (by UV) values for samples from each station and selected capillary GC traces are presented as well.

There were differences in the patterns of accumulation of different aromatic and sulfur heterocyclic hydrocarbons in M. truncata from different water depths in the same bay (e.g., Figure 2.2) and from different stations along the same depth transect (Figure 2.3), perhaps indicating an uneven distribution of hydrocarbons in the bays. In M. truncata from Bays 9 and 10 which received dispersed oil, the compound accumulated to the greatest extent from each of the three homologous series examined in detail was C3-naphthalenes, C2-phenanthrenes and C2-dibenzothiophenes (Figures 2.2 and 2.7). Only very small amounts of higher molecular weight polycyclic aromatic hydrocarbons were accumulated (Σ PAH in figures). On the other hand, M. truncata from Bay 11 which received undispersed oil, preferentially accumulated C4-naphthalenes, C3-phenanthrenes and C 3-dibenzothiophenes. These clams also accumulated proportionately much smaller amounts of naphthalene and alkyl naphthalenes than did clams from Bays 9 and 10. M. truncata from Bay 11 undoubtedly were exposed to more highly weathered oil than clams in Bays 9 and 10.

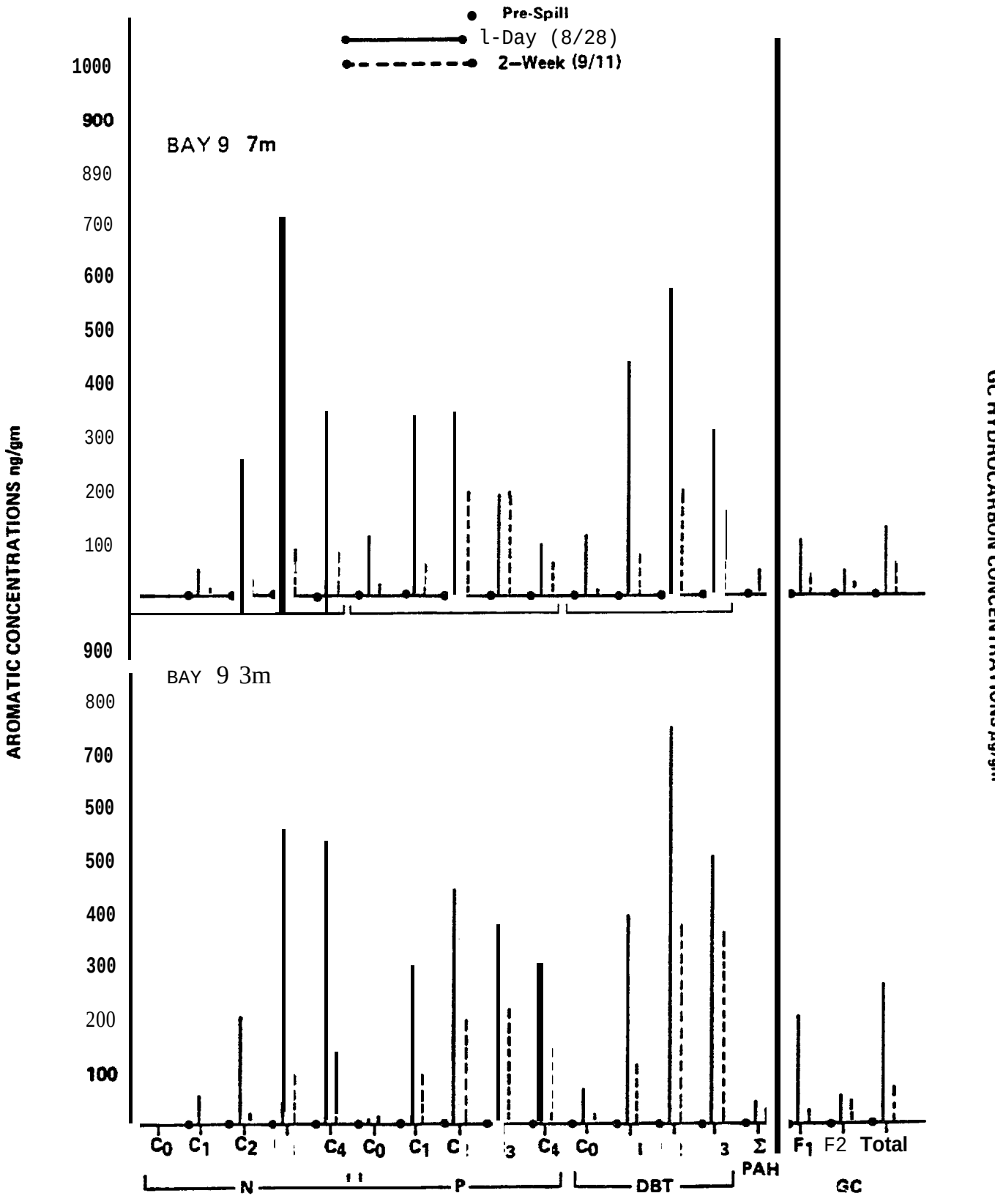

Figure 2.2. **Mya aromatic profiles (by GC2/MS),** (Bay 9).

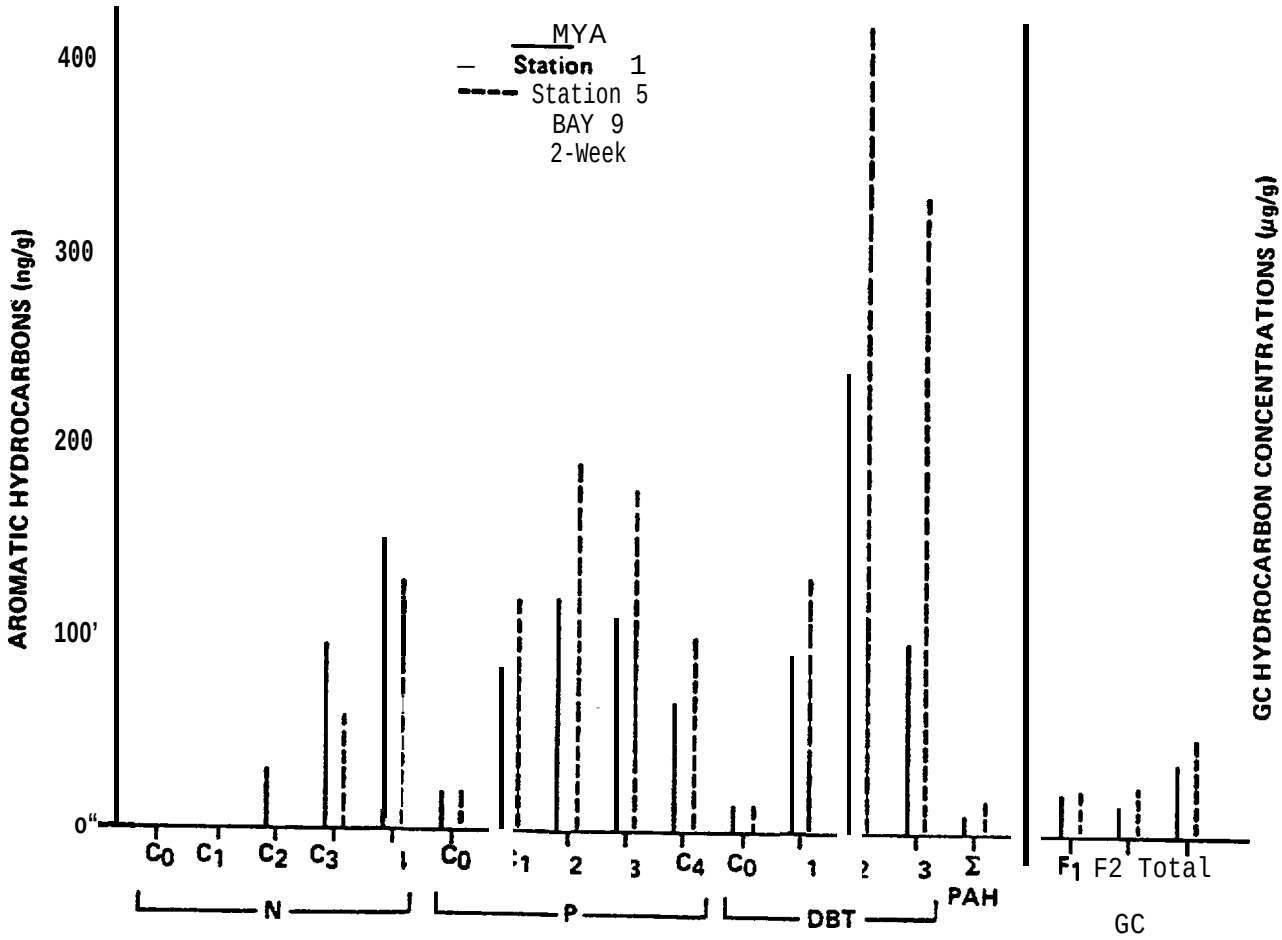

Figure 2.3. **Variation of aromatic hydrocarbon** levels in **Mya** along 7 meter depth stratum (Bay 9).

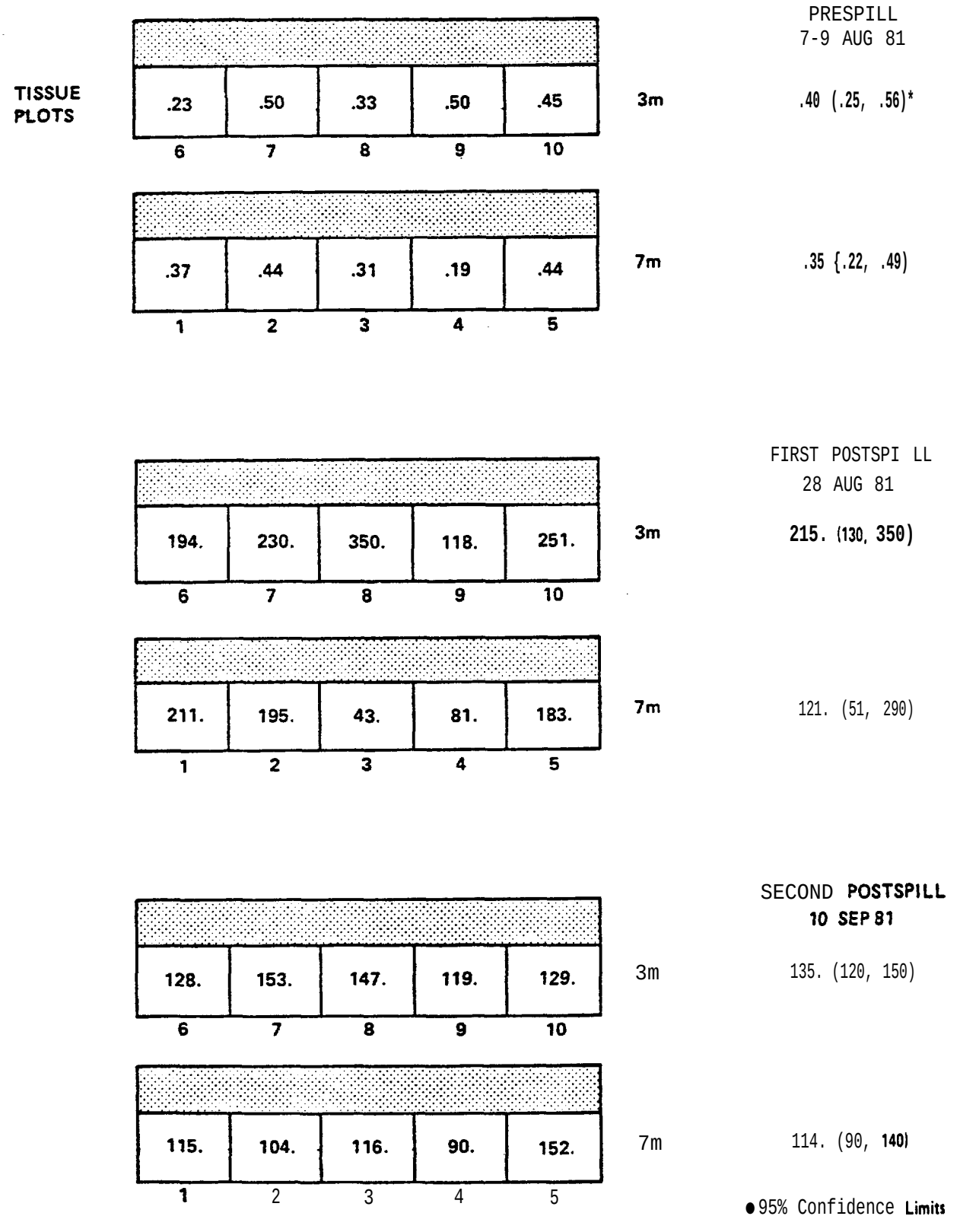

Figure 2.4. Concentrations of oil in Mya truncata, Bay 9 by UV/F (µg/g).

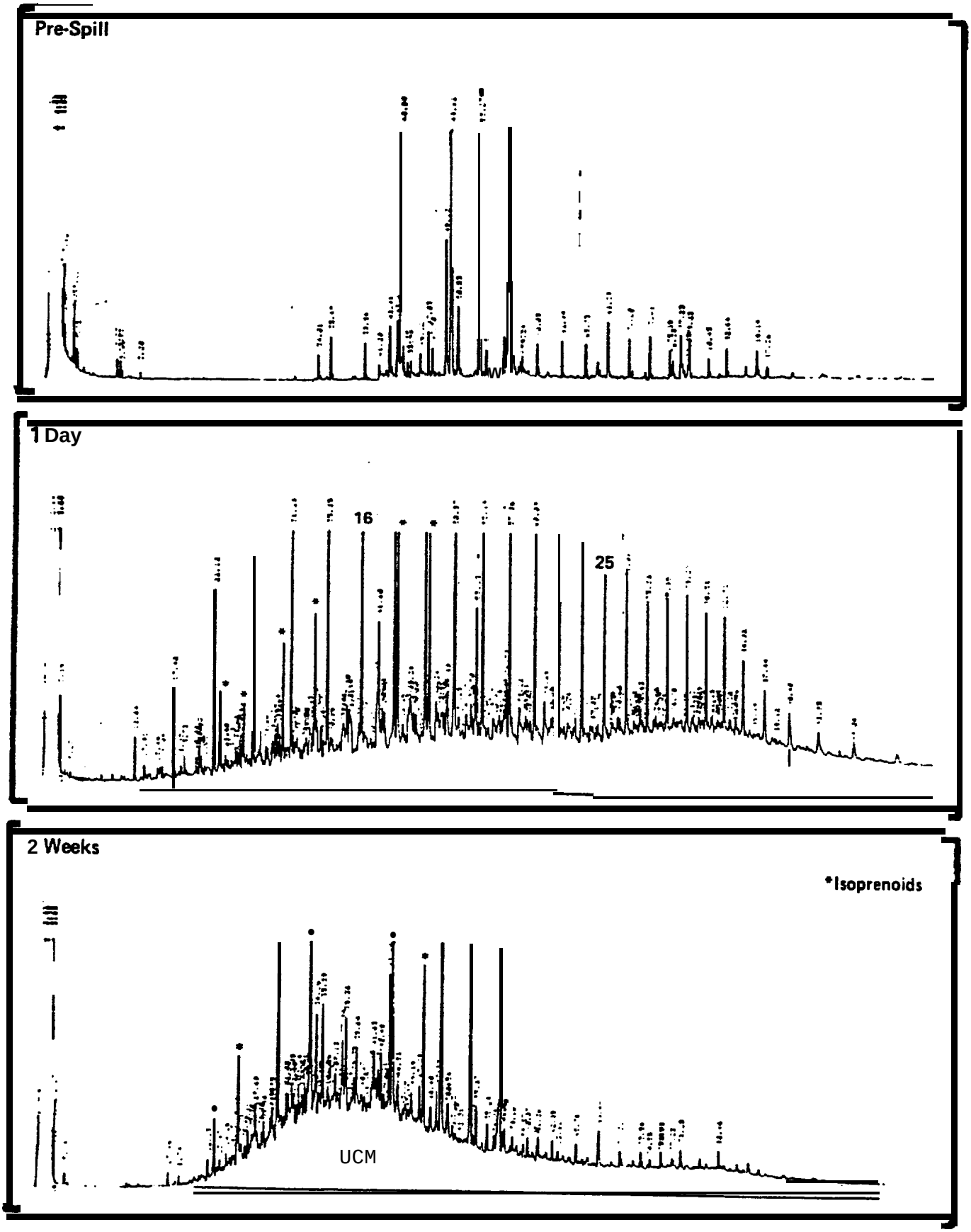

Figure 2.5. Mya truncata-GC2 profiles of Bay 9 animals (saturated hydrocarbons).

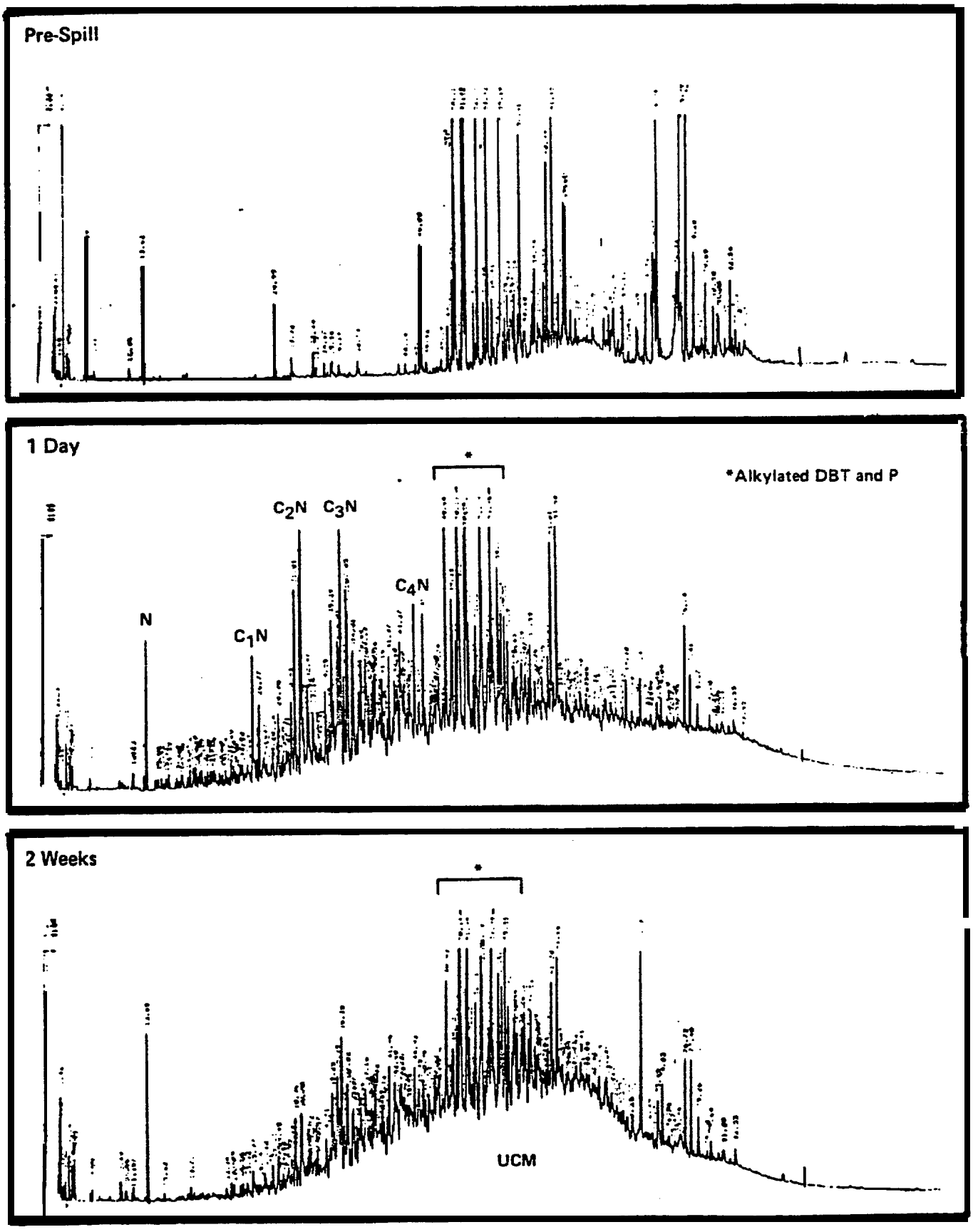

Figure 2.6. **Mya truncata-GC2profiles of Bay 9 animals (aromatics).**

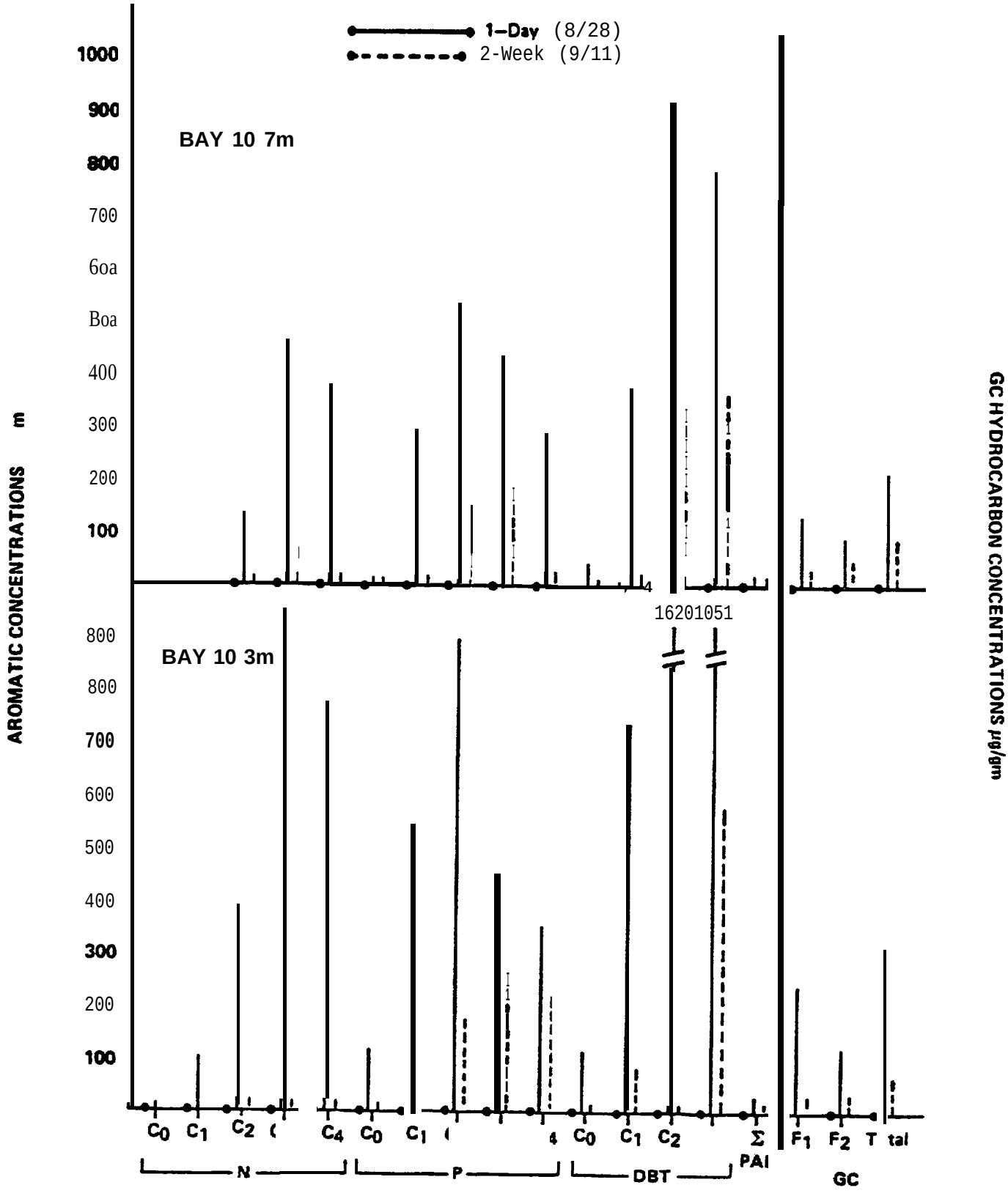

Figure 2.7. **Aromatic profiles from Mya exposed to oil, illustrating changes in concentrations over 2 weeks (Bay 10).**

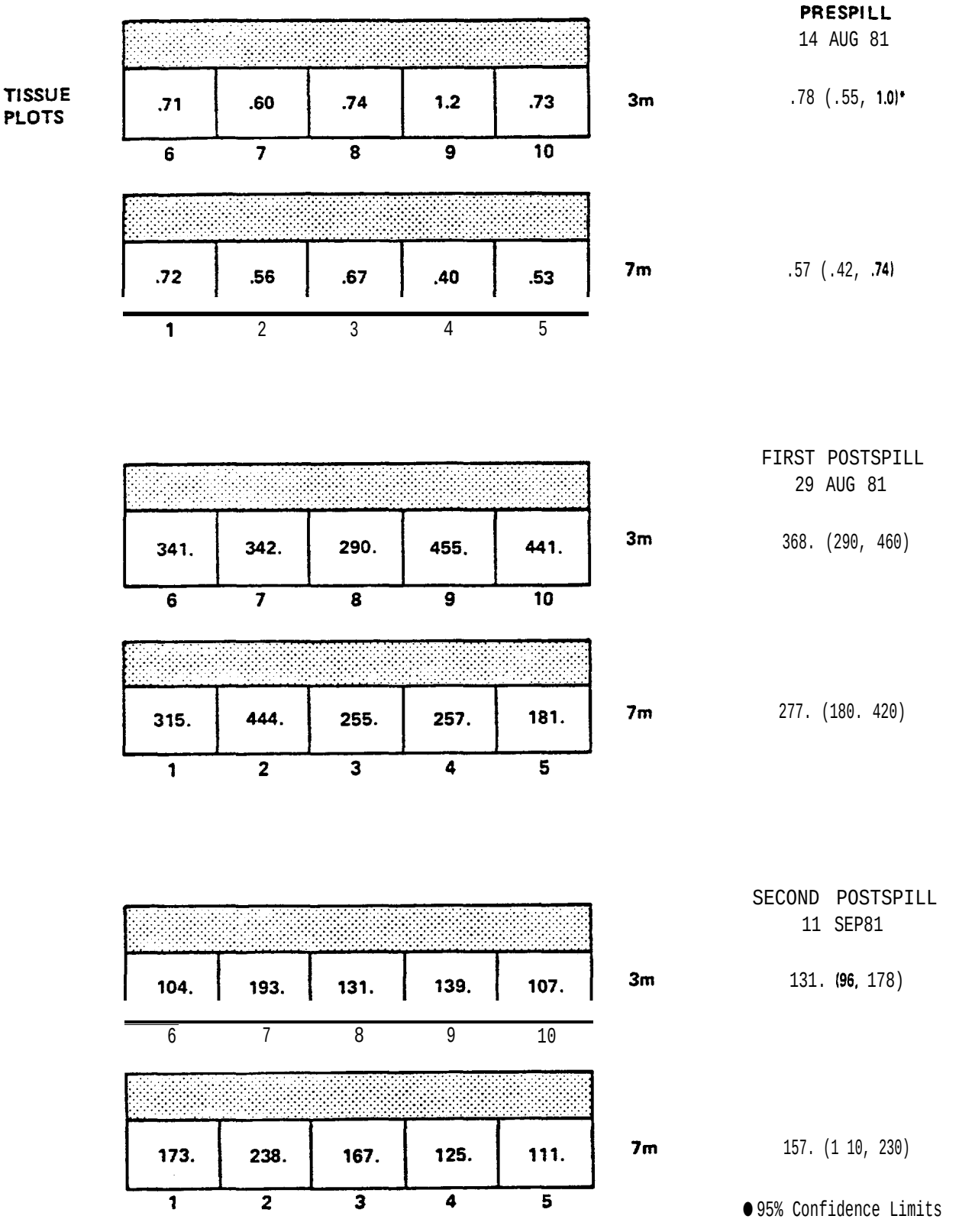

Figure 2.8. Concentrations of oil in Mya truncata, Bay 10 by UV/F (µg/g).

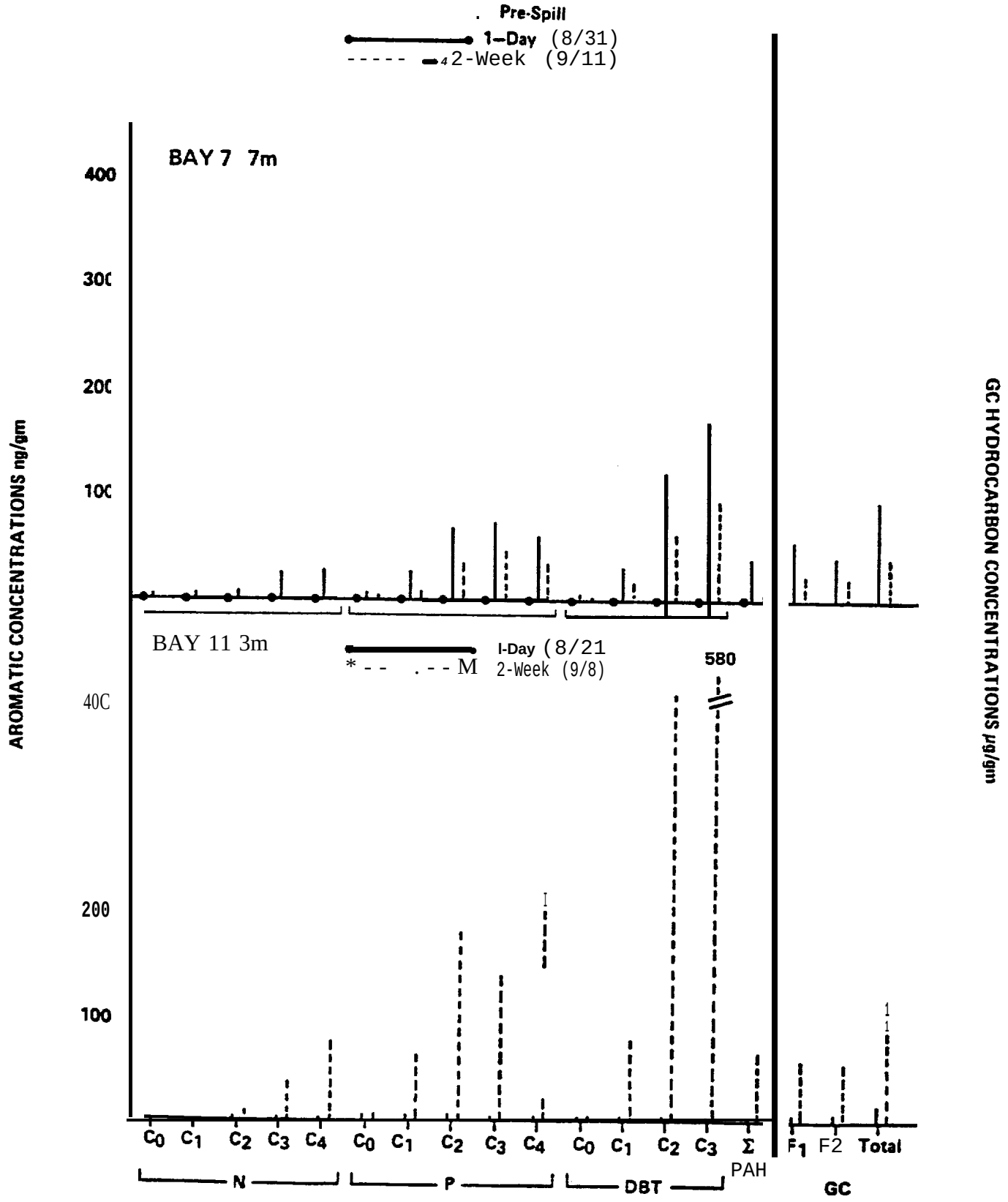

Figure 2.9. Mya truncata; aromatic profiles of Bays 7 & 11 by GC2/MS.

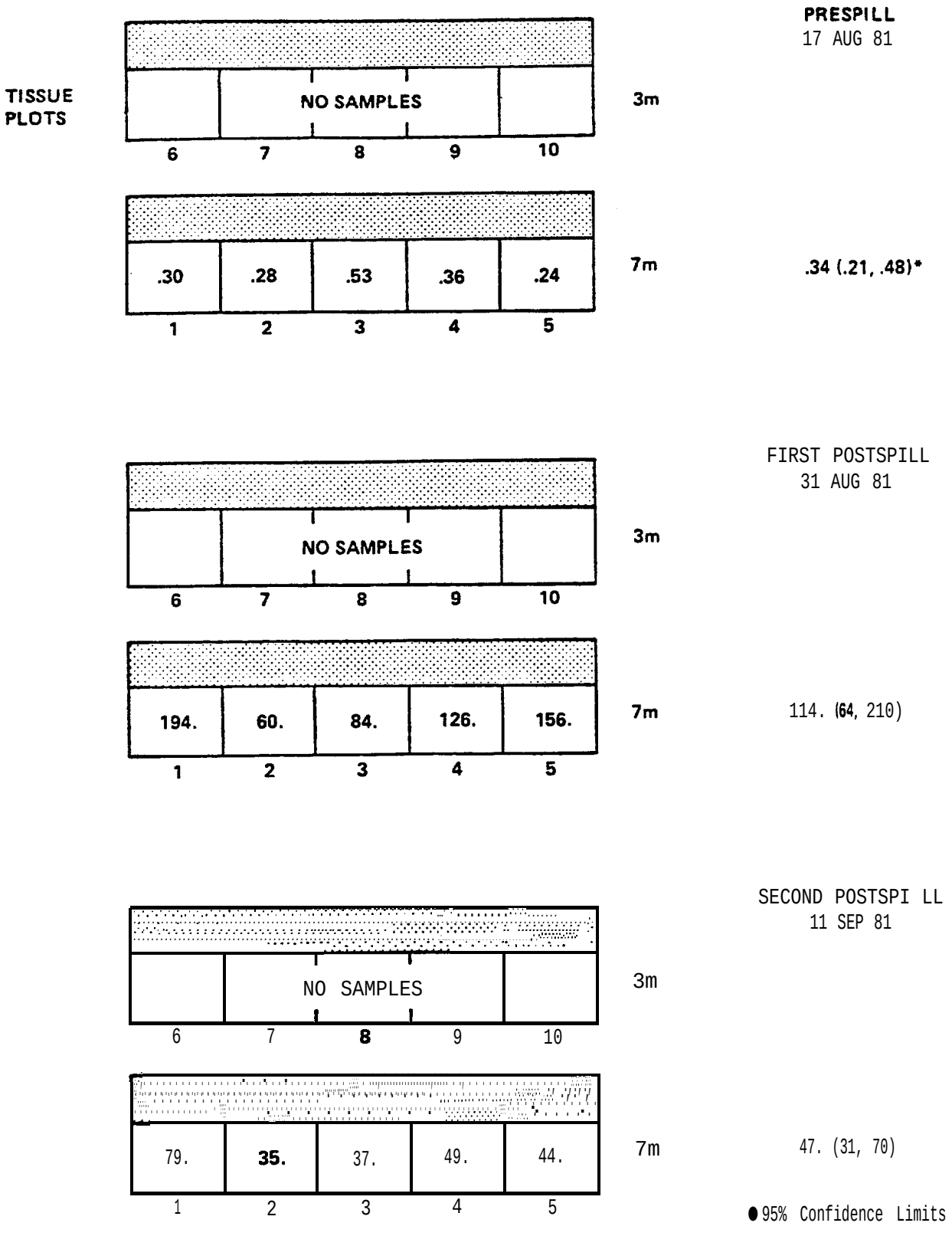

Figure 2.10. **Concentrations of oil in** Mya truncata, **Bay 7 UV/F (µg/g).**

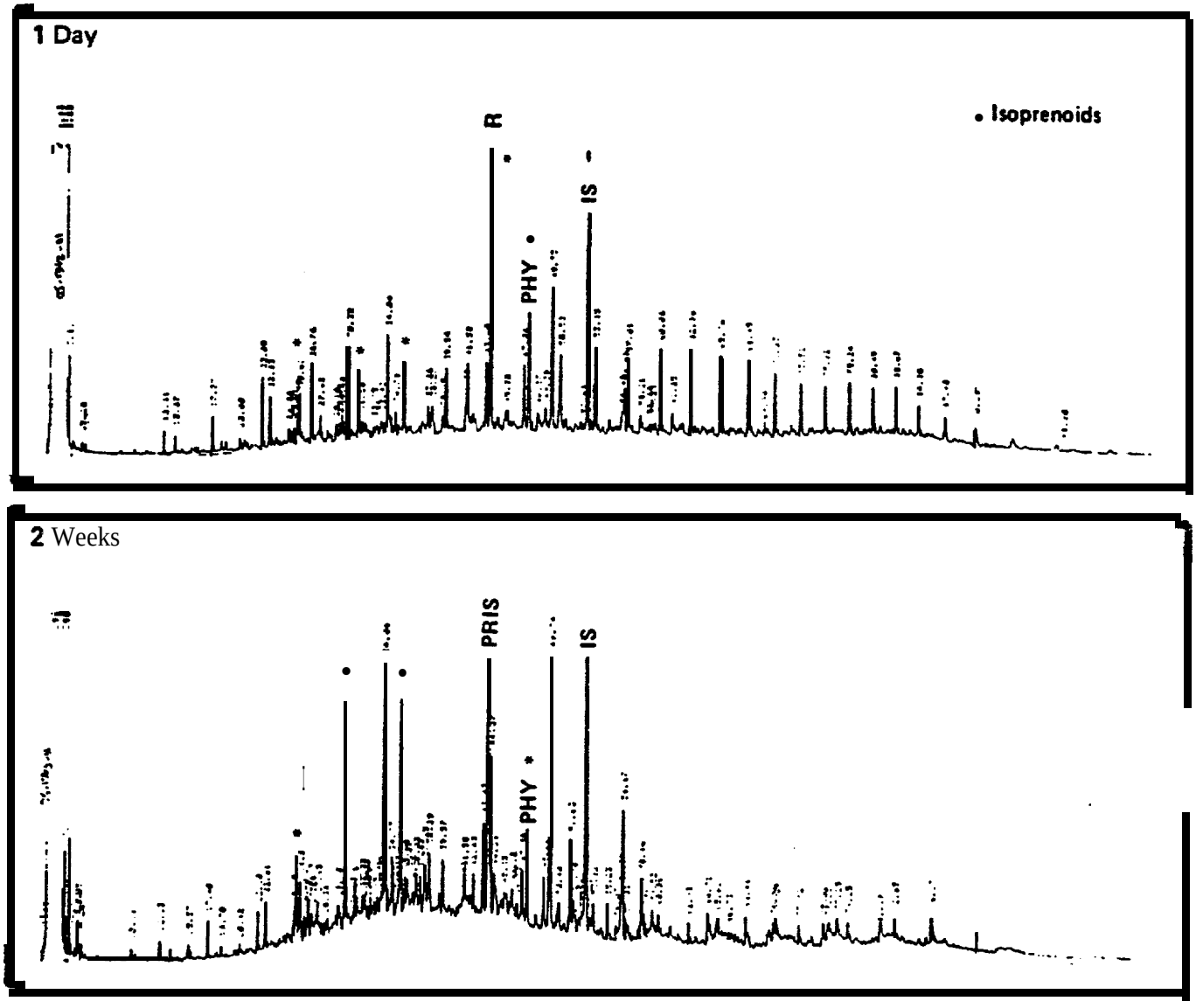

Figure 2.11. Mya truncata-GC2 profiles of Bay 7 animals (saturates).

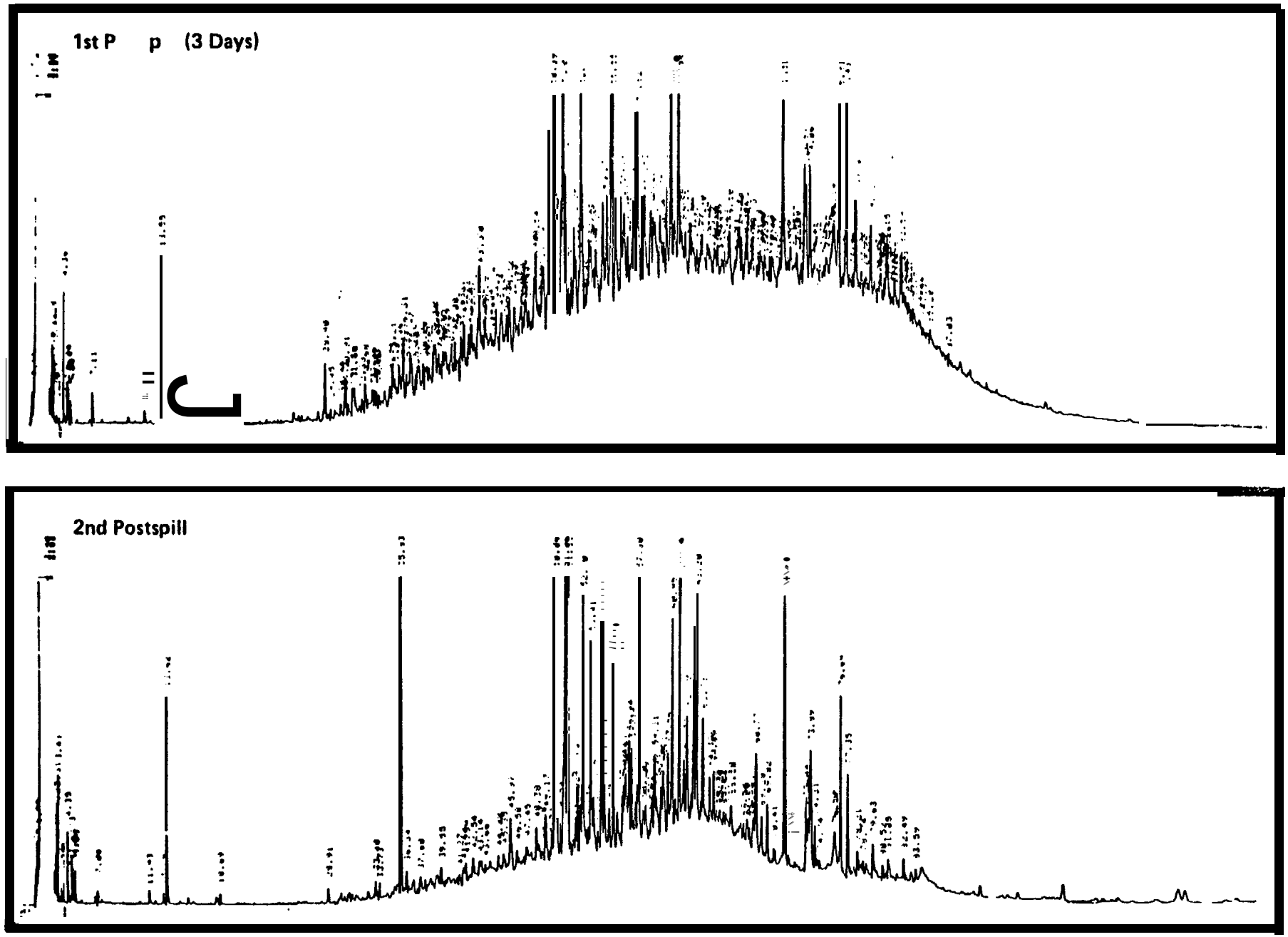

Figure 2.12. Mya truncata-GC[2] profiles of Bay 7 animals (aromatics).

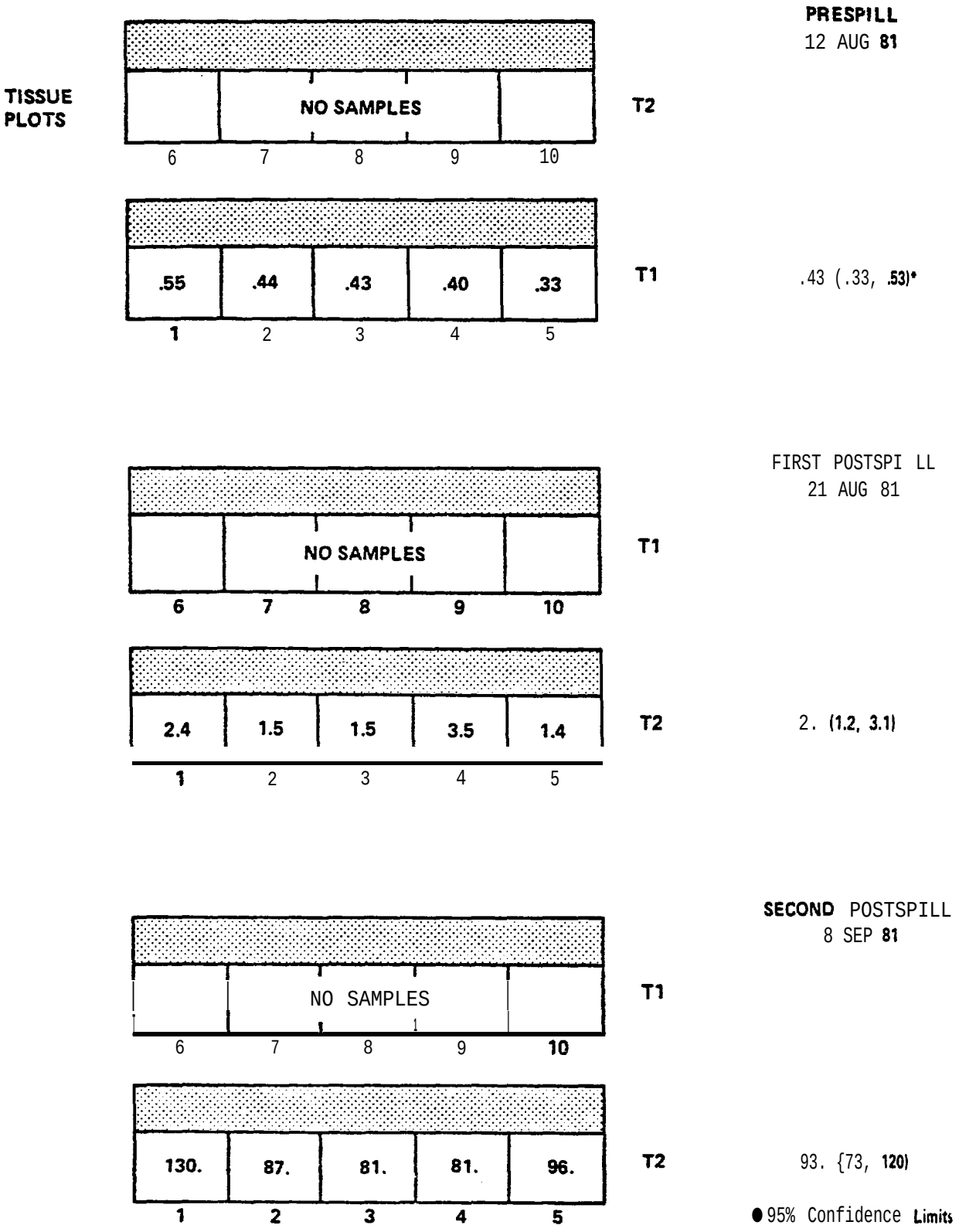

Figure 2.13. Concentrations of oil in Mya truncata, Bay 11 by UV/F (µg/g).

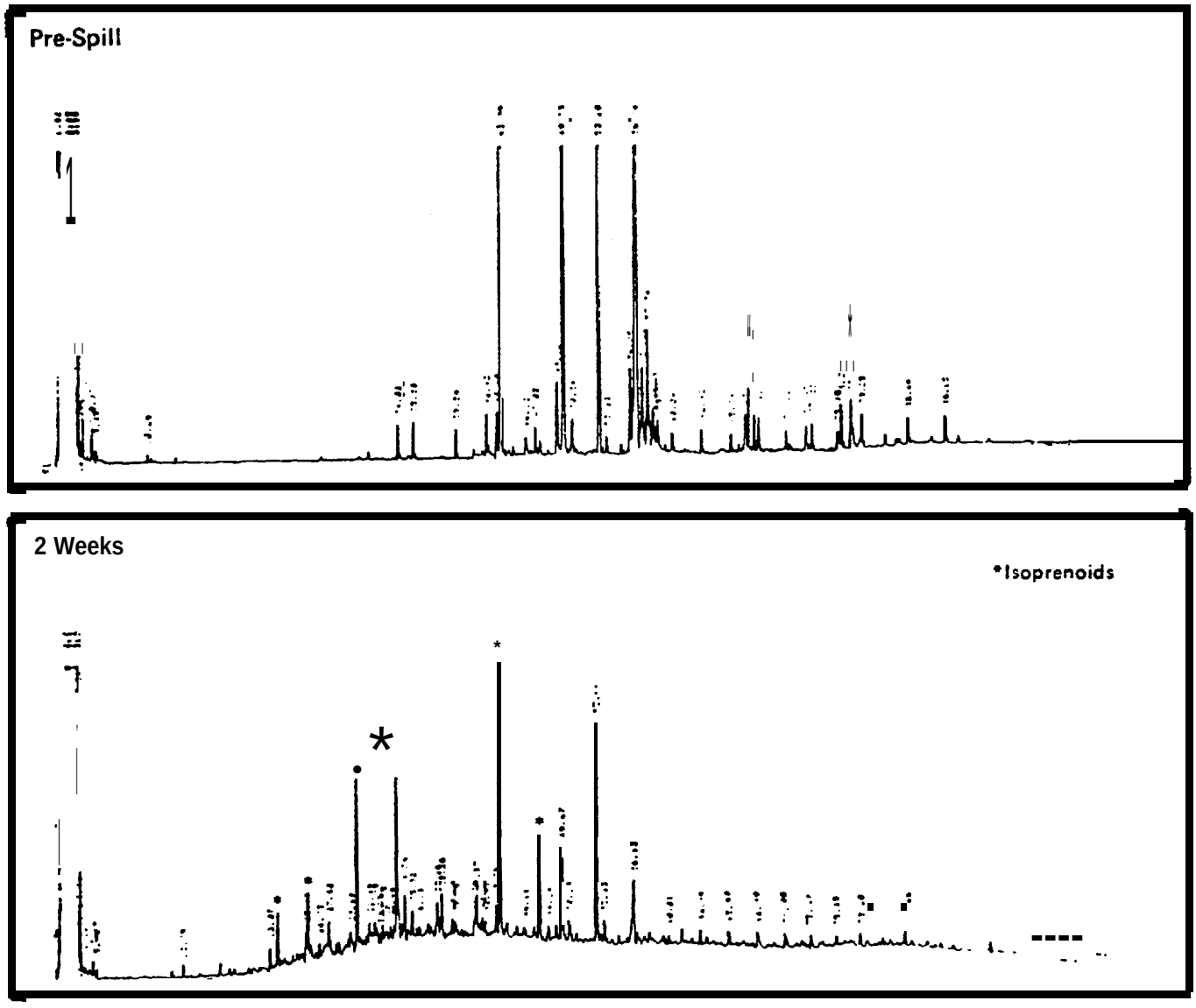

Figure 2.14. Mya truncata-Bay 11 (saturates).

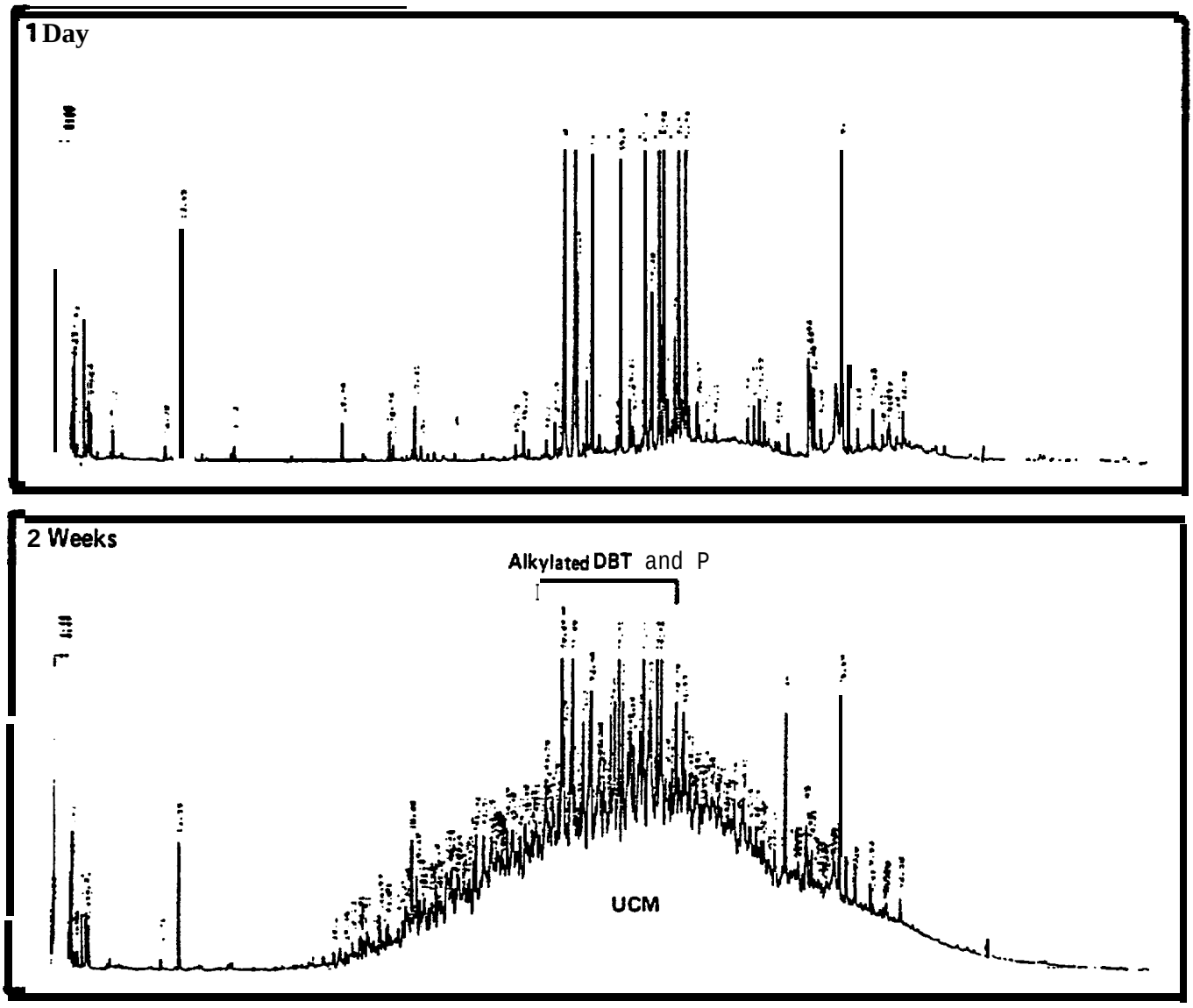

Figure 2.15. **Mya truncata-Bay 11 (aromatics).**

In all cases but one (Bay 10, second post-spill sample) where comparisons could be made, clams from the 3-meter water depth contained higher concentrations of total hydrocarbons than clams collected at the same time from the 7-meter water depth.

2.2.2. **Serripes groenlandicus.** Seventeen samples of Serripes groenlandicus were analyzed. These include the pre-spill, l-day post-spill, and 2-week post-spill samples from Bays 7, 9, and 10(7 meters), the l-day and 2-week post-spill samples from Bay 11 (7 meters), the 3-meter sample set from Bay 9, and the analyses of two individual stations along the 7-meter depth stratum in Bay 9. Additionally, we had the opportunity to analyze the gut of a 1-day post-spill Serripes collection separately from the remaining tissue to examine chemical differences within the animals. Results of GC/MS/DS analyses of aromatic and sulfur heterocyclic hydrocarbons, total petroleum (by UV) values, and capillary GC traces are presented in Figures 2.16-2.29.

In general, the aromatic/heterocyclic hydrocarbon profiles in tissues of S. groenlandicus are quantitatively similar to those in Mya truncata. In a sample of S. groenlandicus collected from Bay 10 immediately after the spill, concentrations of alkyl benzenes were higher in muscle tissue than in gut tissue (Figure 2.24). Concentrations of phenanthrenes, dibenzothiophenes and total higher molecular weight polycyclic aromatic hydrocarbons were higher in gut tissue than in muscle tissue.

2.2.3. **Astarte borealis.** Eight samples of Astarte borealis were analyzed. These include samples from the 7-meter depth stratum from Bays 9, 10, 11, and 7 during the first and second post-spill samplings (i.e., l-day, 2-weeks). Results of GC/MS/DS analysis of aromatic/heterocyclic hydrocarbons, total petroleum concentration information, and representative GC traces are summarized in Figures 2.30-2.37.

A. borealis from Bays 9 and 10 accumulated much higher concentrations of aromatic and heterocyclic hydrocarbons, particularly immediately after the oil spill, than did Mya truncata and Serripes groenlandicus. The dominant hydrocarbons in tissues of A. borealis from these two bays were C_3-C_4-naphthalenes, C_1-C_3-phenanthrenes and C_1-C_3-dibenzothiophenes (Figure 2.30). A. borealis from Bay 11 contained proportionately lower concentrations of C_1-phenanthrenes and C_1-dibenzothiophenes than did animals from Bays 9 and 10.

2.2.4. **Nitrogen heterocyclics.** Four pooled sample extracts were processed and analyzed by GC/MS/DS to determine the presence, identity, and concentration of the basic PANH compounds. Samples analyzed were:

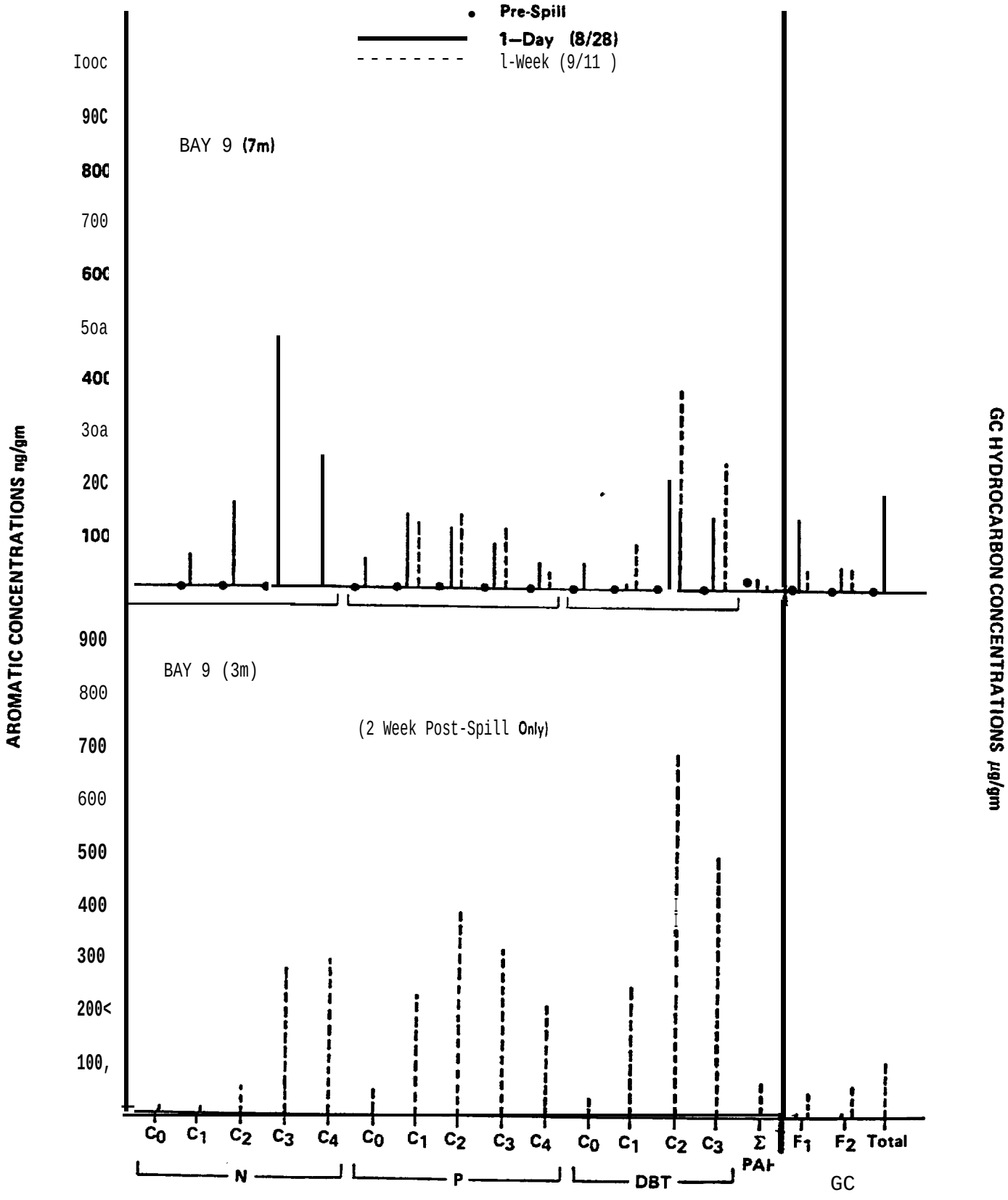

Figure 2.16. Serripes aromatic profiles (by GC2/MS), (Bay 9).

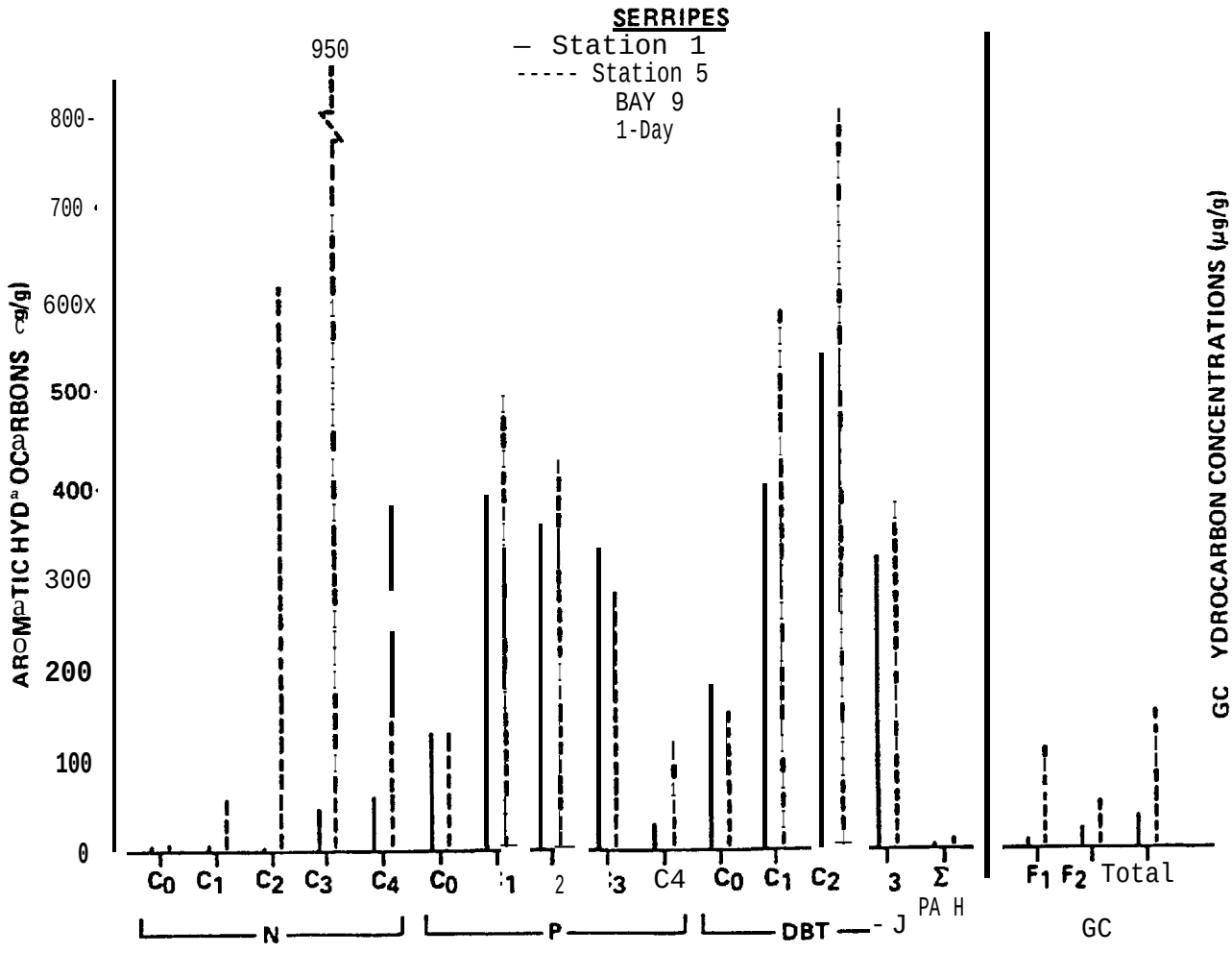

Figure 2.17. Variation of aromatic hydrocarbon levels in Serripes along 7 meter depth stratum (Bay 9).

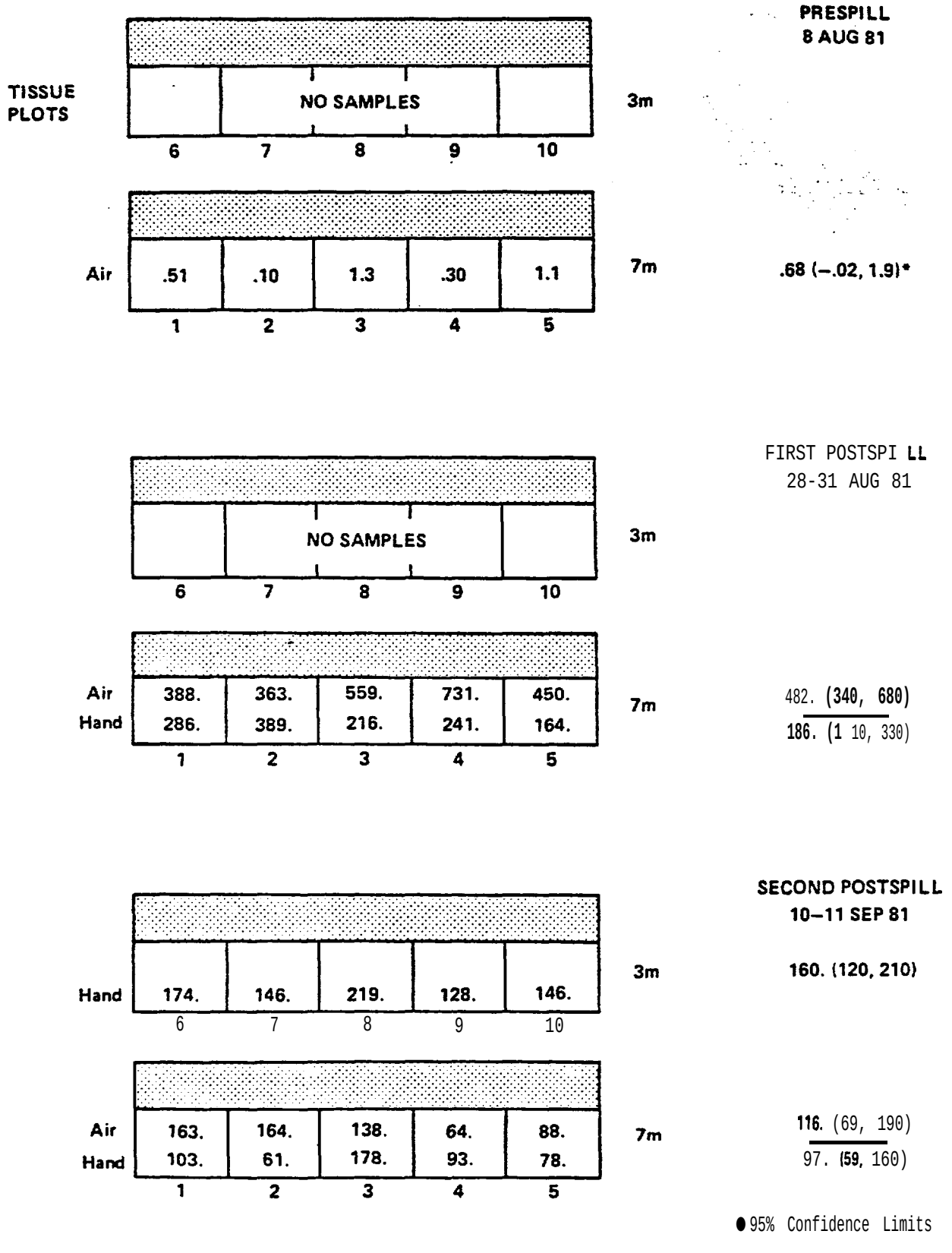

Figure 2.18. Concentrations of oil in Serripes, Bay 9 by W/F (µg/g).

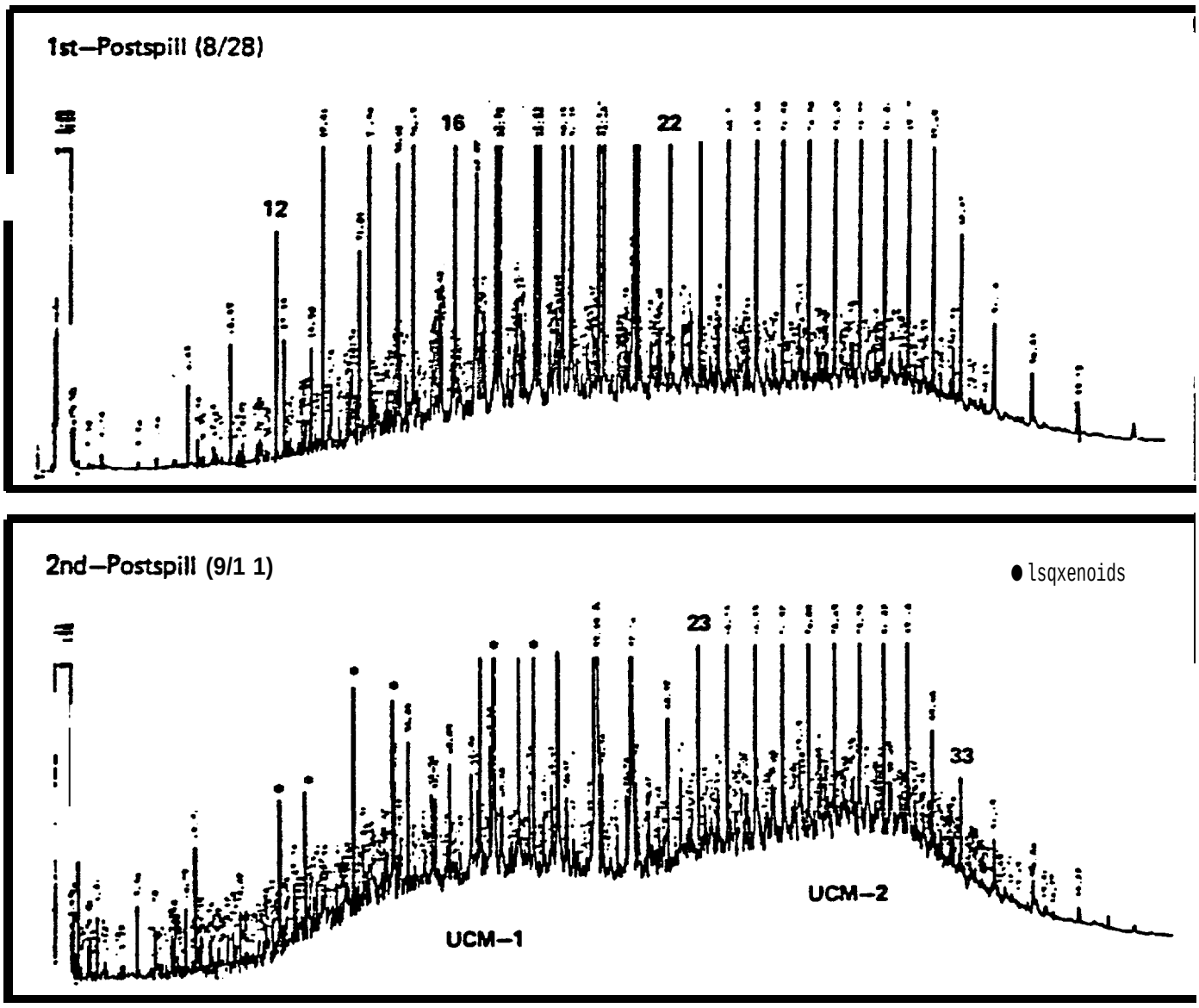

Figure 2.19. **Serripes groenlandicus-GC2 profiles of Bay 9 animals (saturates).**

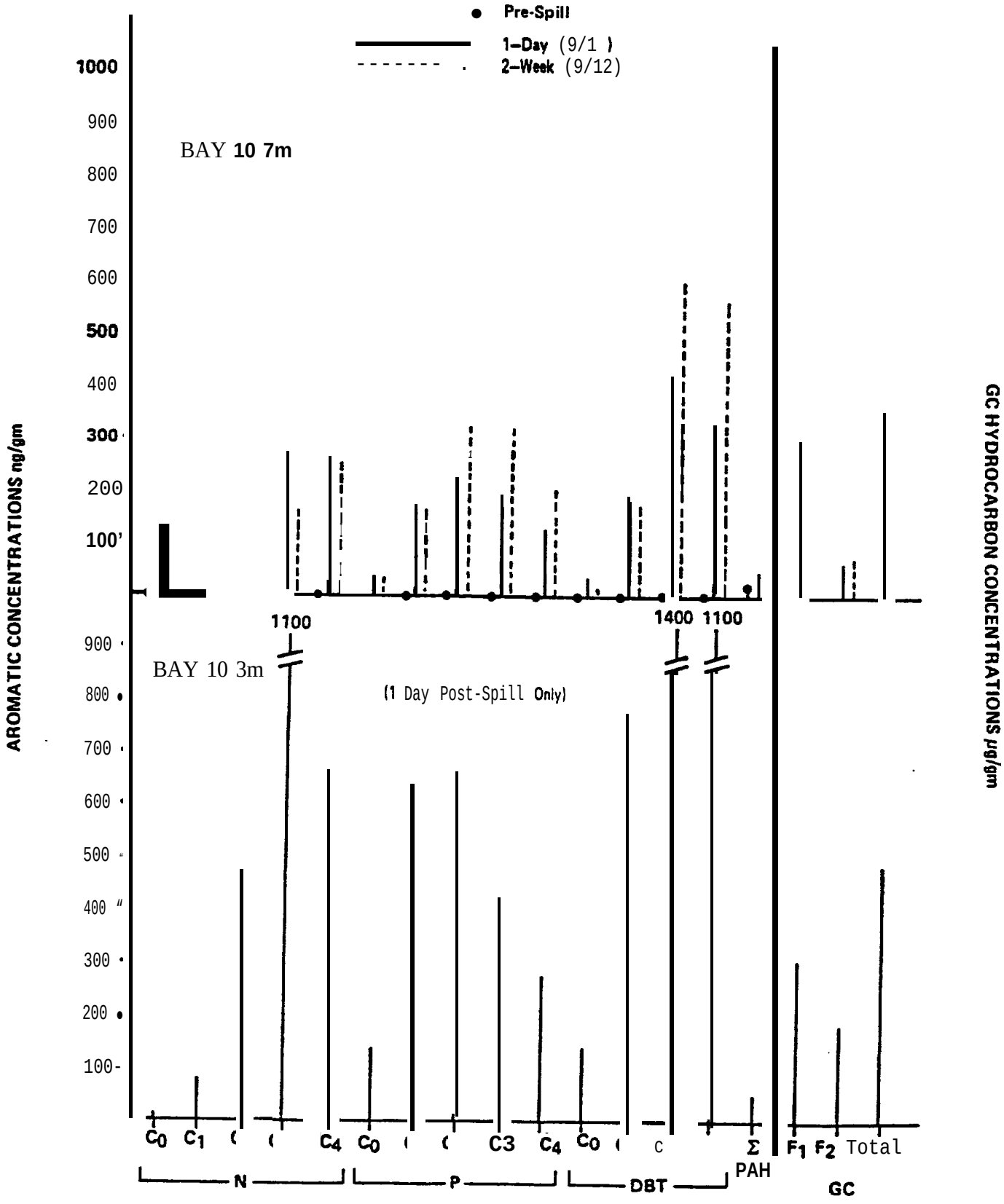

Figure 2.20. **Serripes aromatic profiles, (Bay** lo).

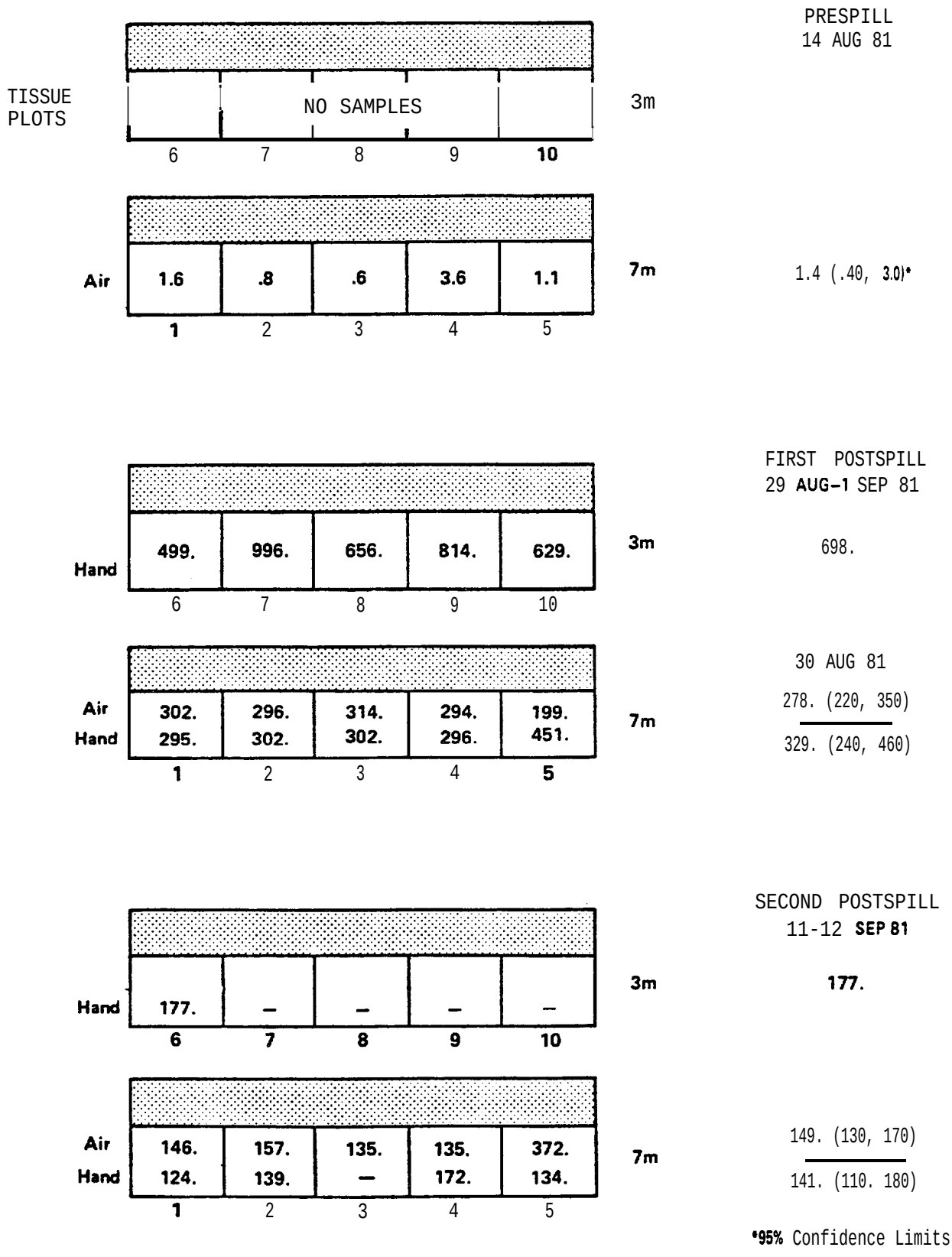

Figure 2.21. Concentrations of oil in Serripes, Bay 10 by UV/F (µg/g).

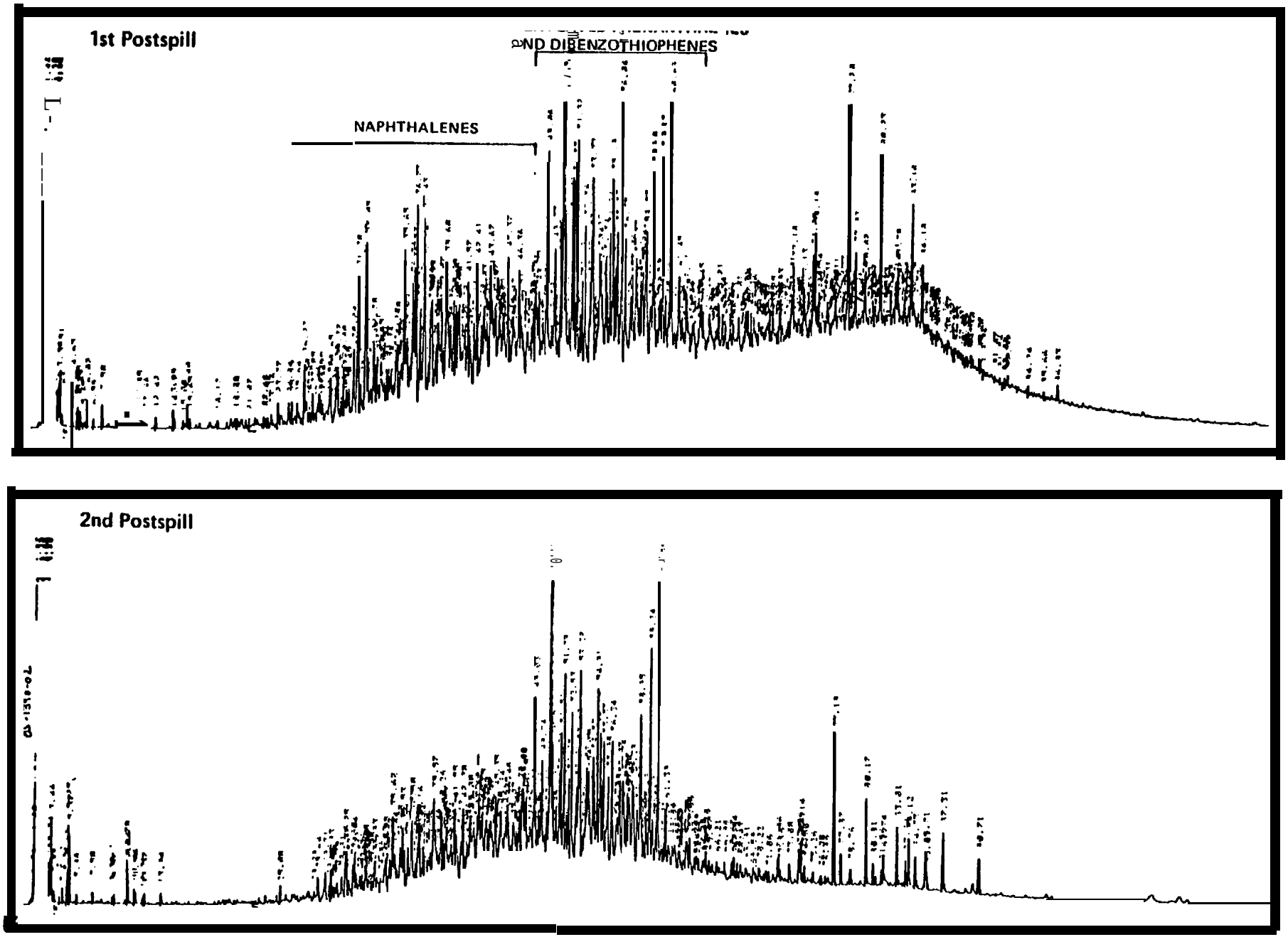

Figure 2.22. Aromatic hydrocarbons in Serripes-Bay 10, (3 meters).

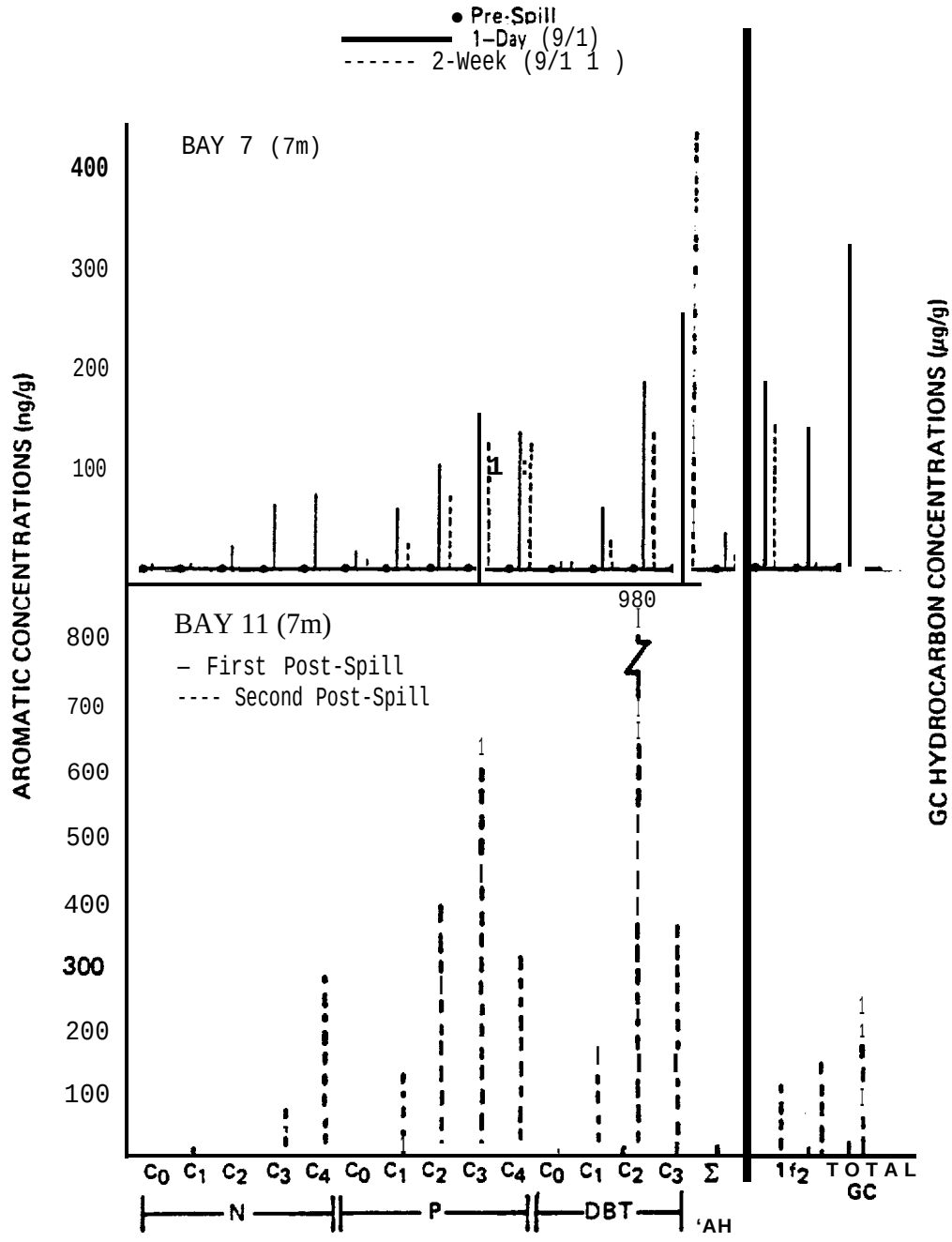

Figure 2.23. Aromatic hydrocarbon profiles in Serripes by GC2/MS (Bay 7 and 11).

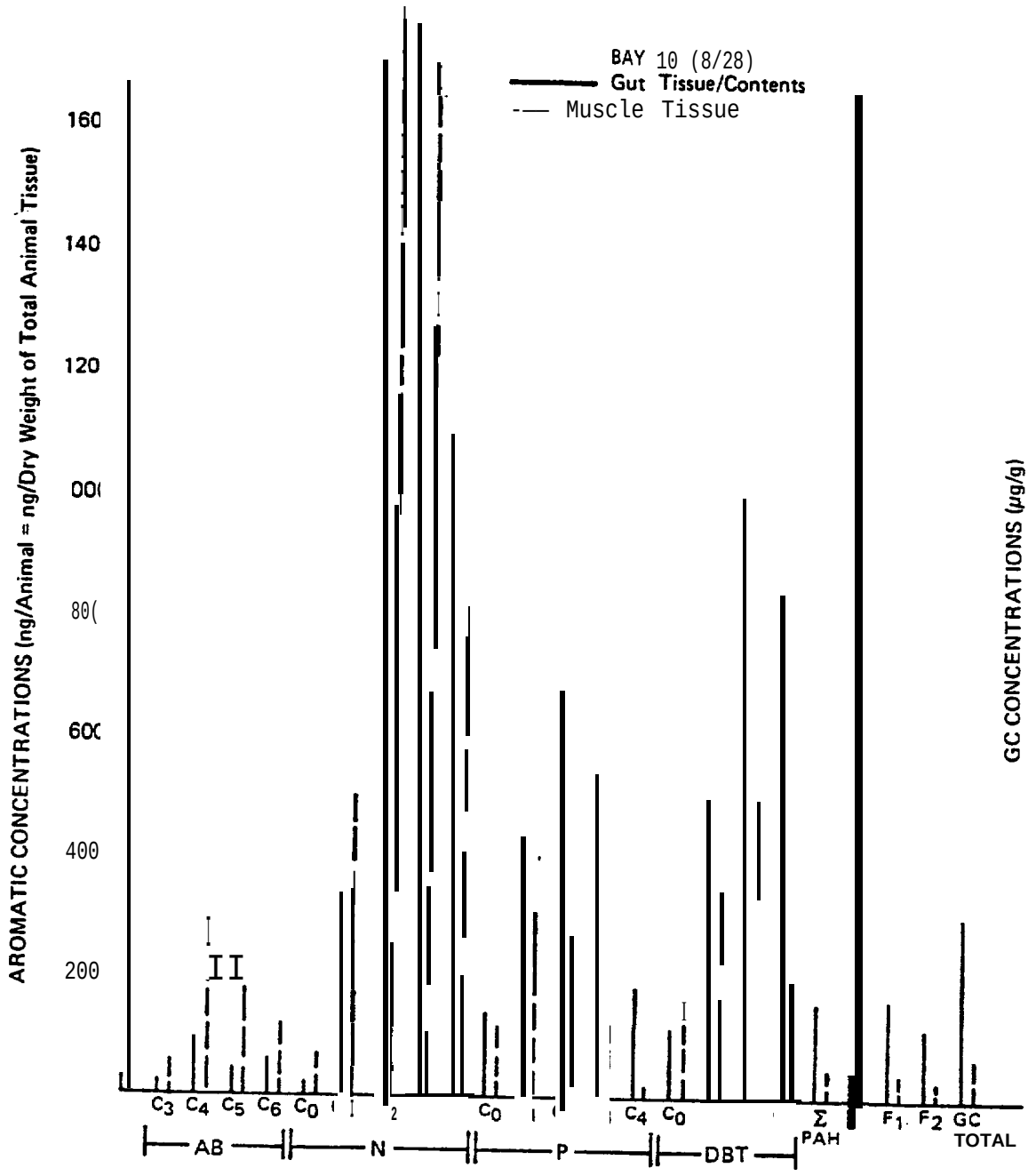

Figure 2.24. Aromatic hydrocarbon profiles of Serripes parts.

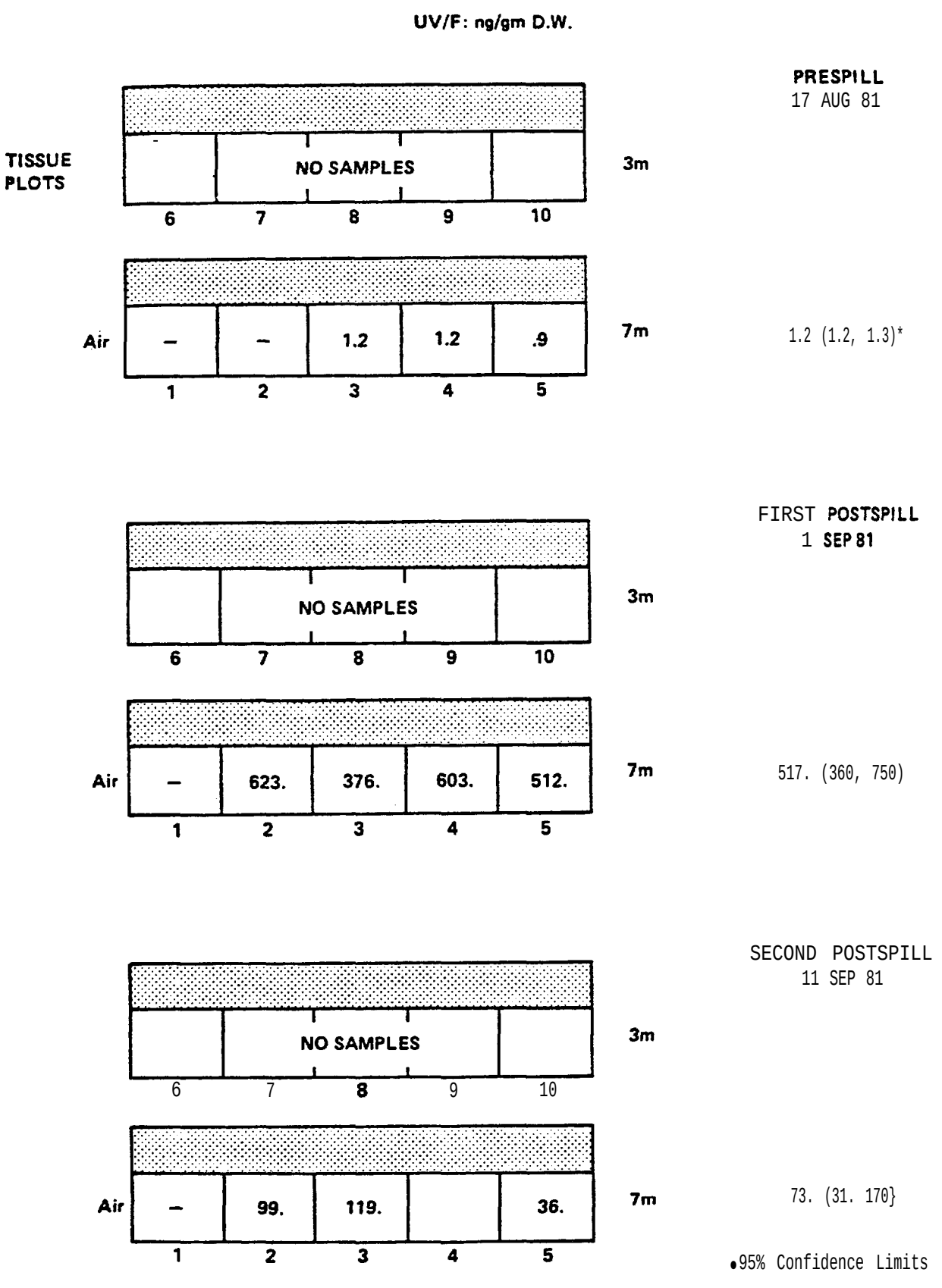

Figure 2.25. Concentrations of oil in Serripes, Bay 7 by UV/F (µg/g).

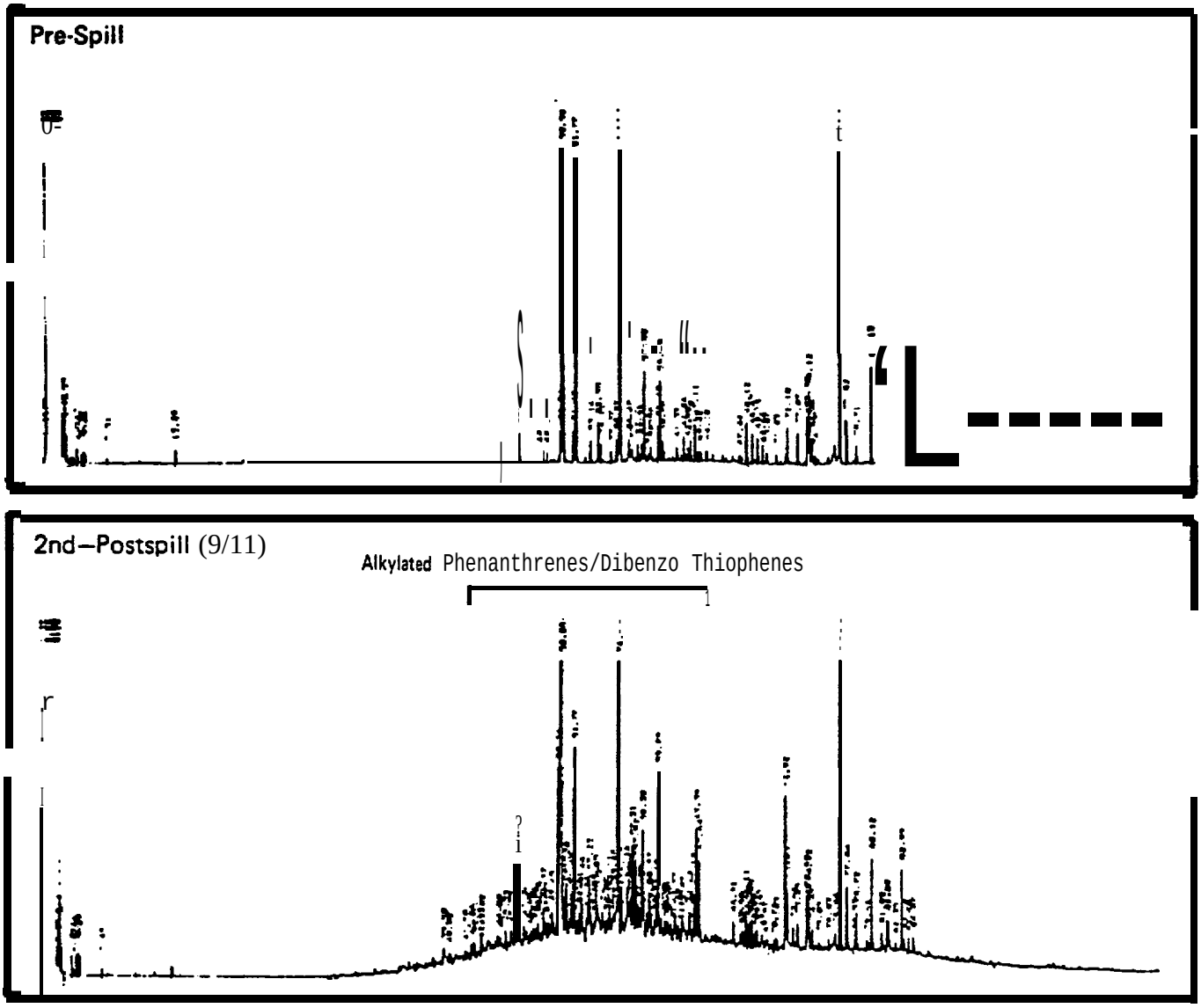

Figure 2.26. **Serripes Bay** 7 **(aromatics).**

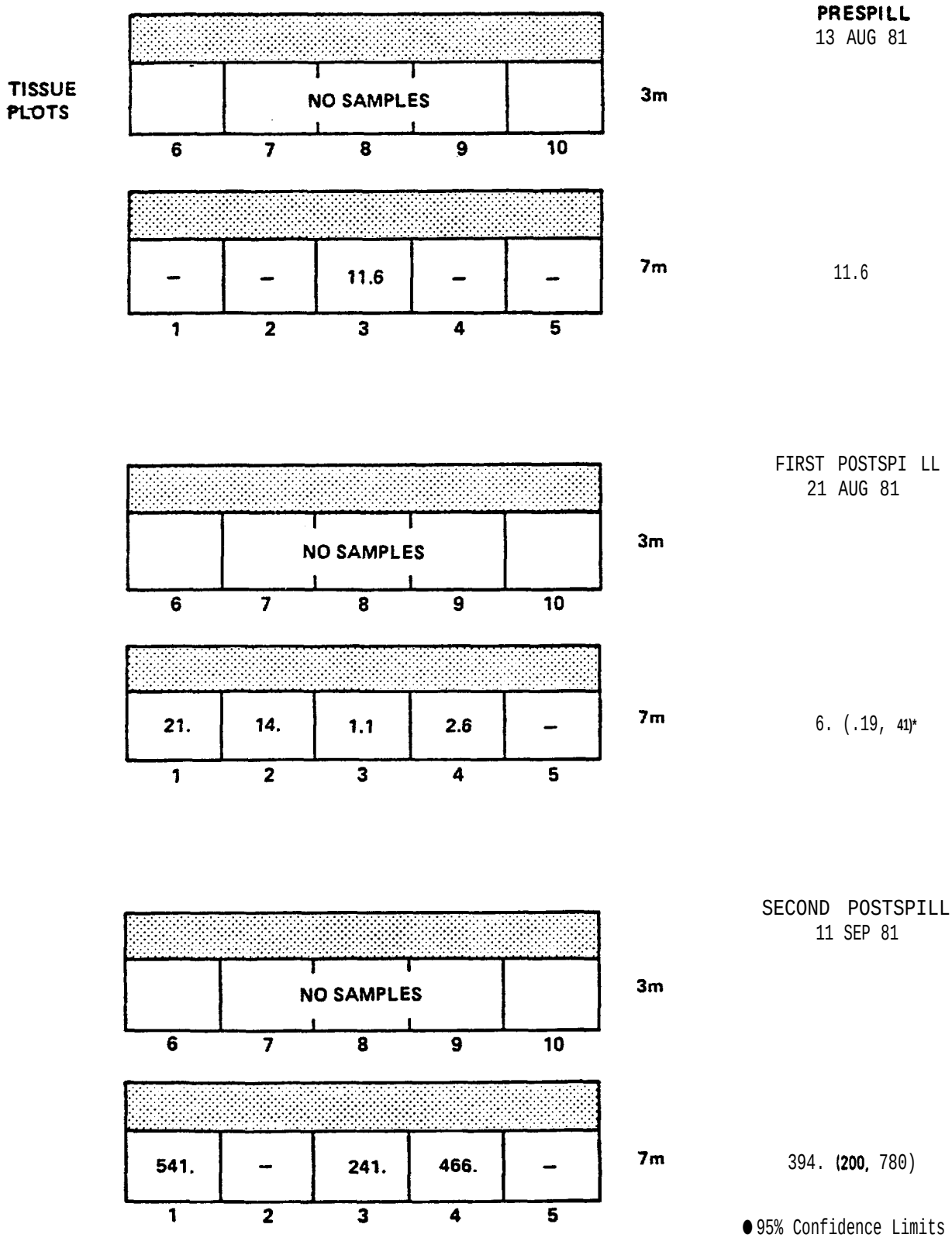

Figure 2.27. Concentrate-of oil in Serripes, Bay 11 by UV/F (µg/g).

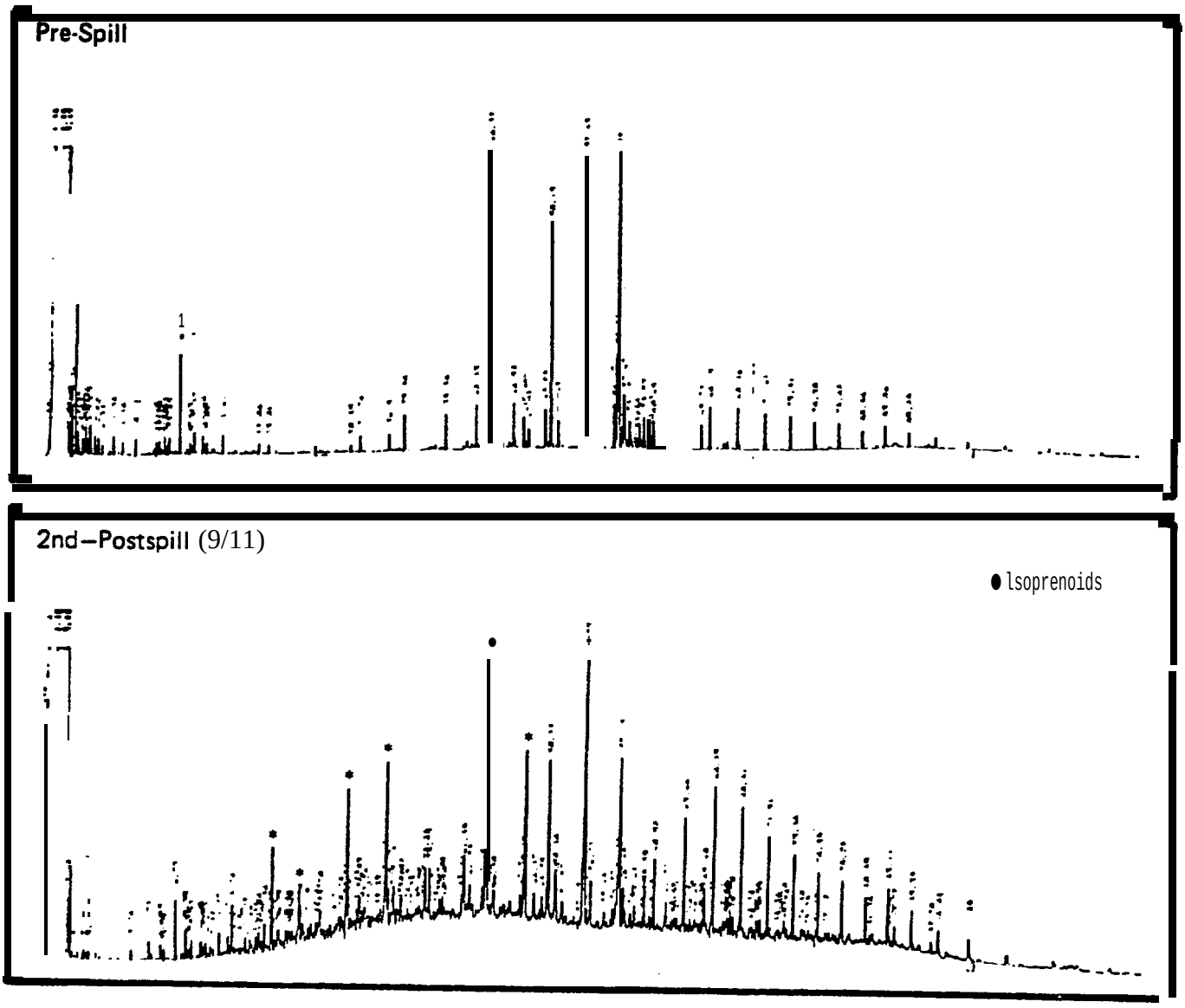

Figure 2.28. Serripes-Bay 1 I (saturates).

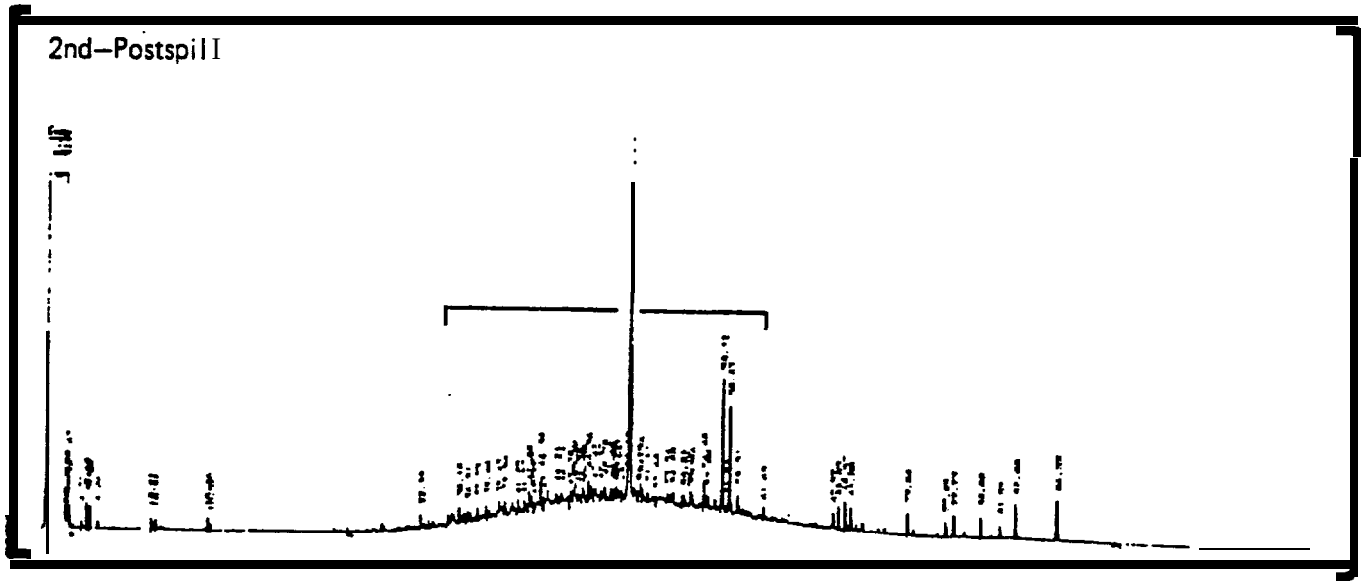

Figure 2.29. Serripes-Bay 11 (aromatics).

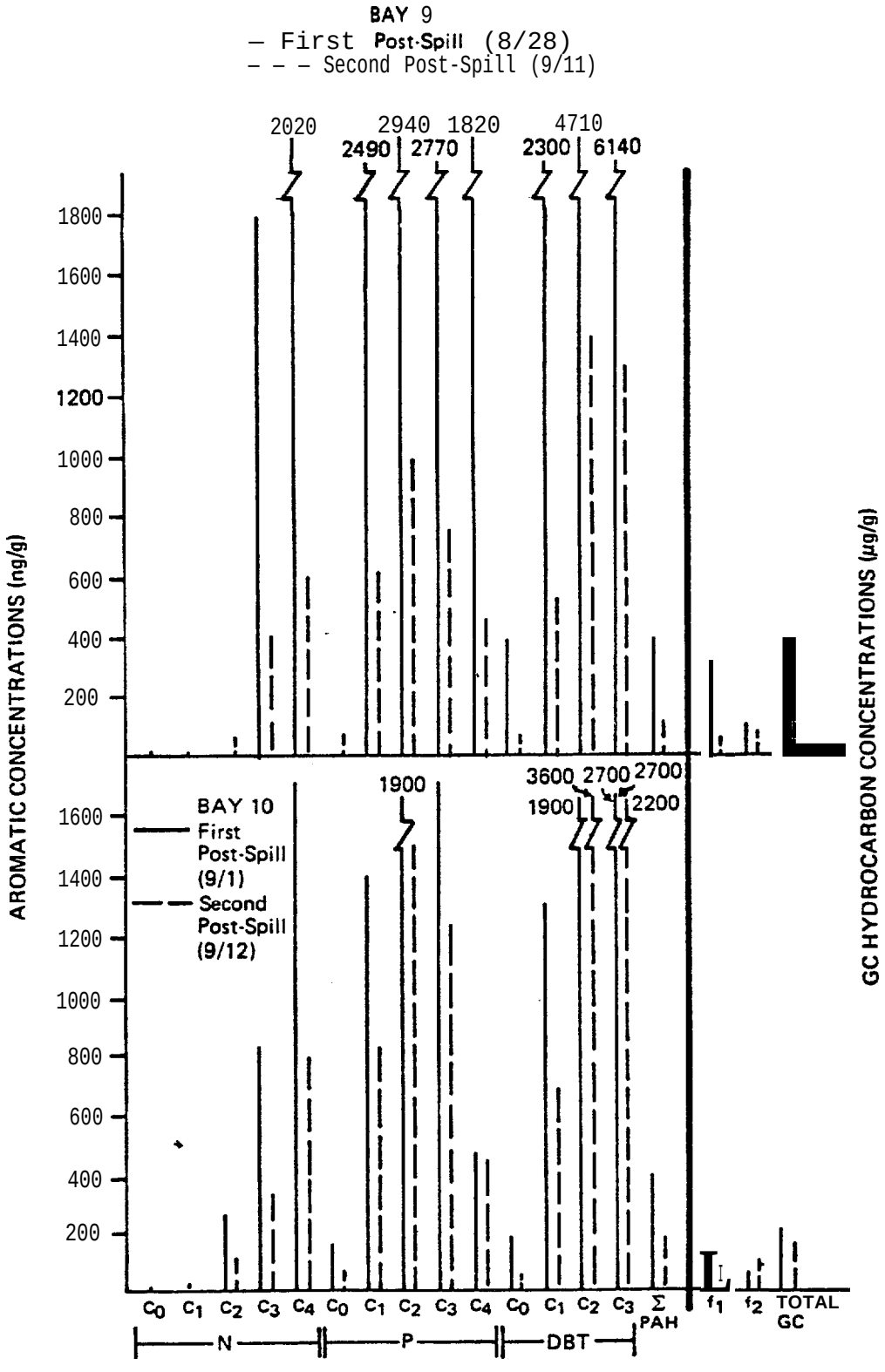

Figure 2.30. **Astarte aromatic profiles (Bays** 9 **and 10).**

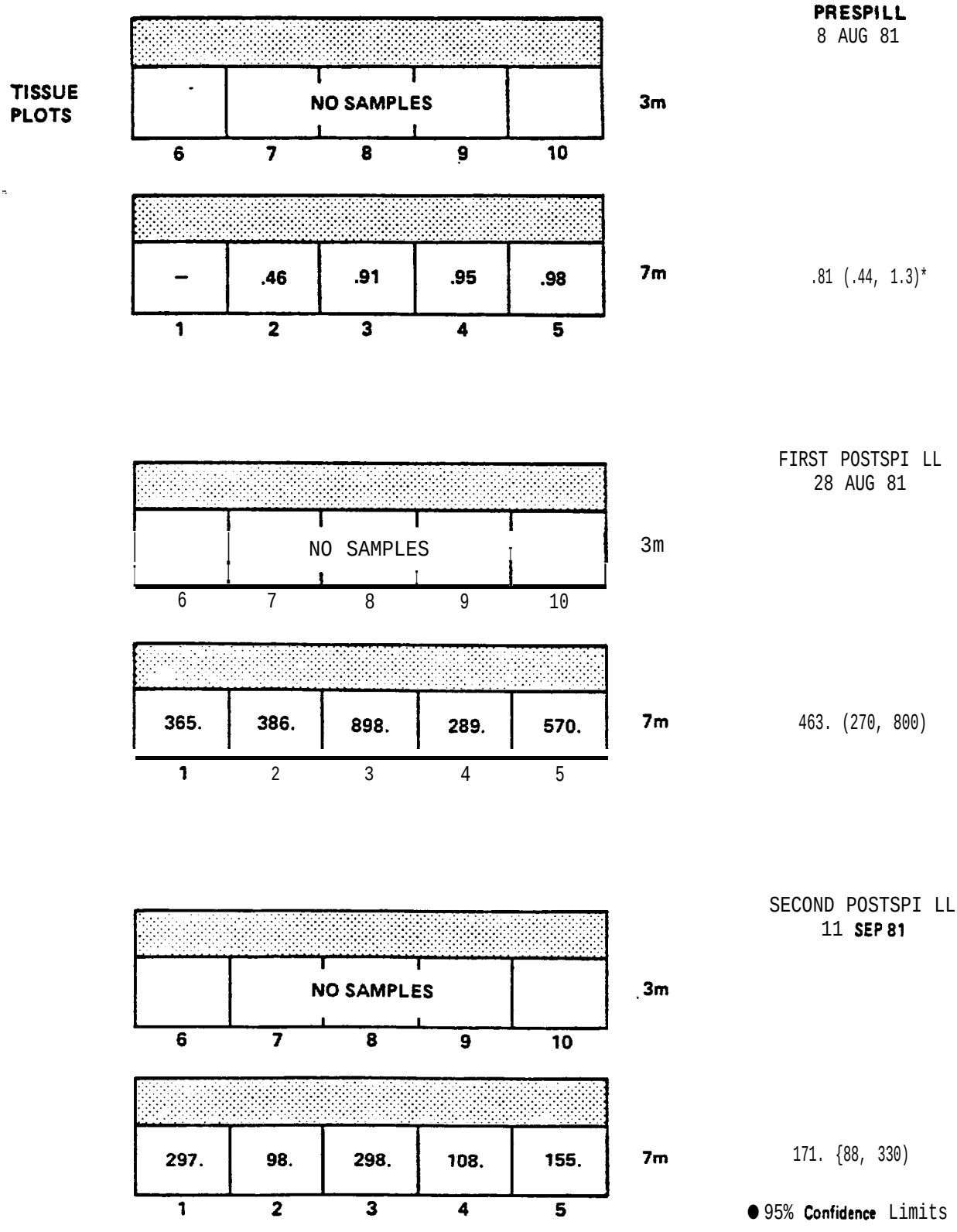

Figure 2.31. Concentrations of oil in Astarte borealis, Bay 9 by UV/F (µg/g).

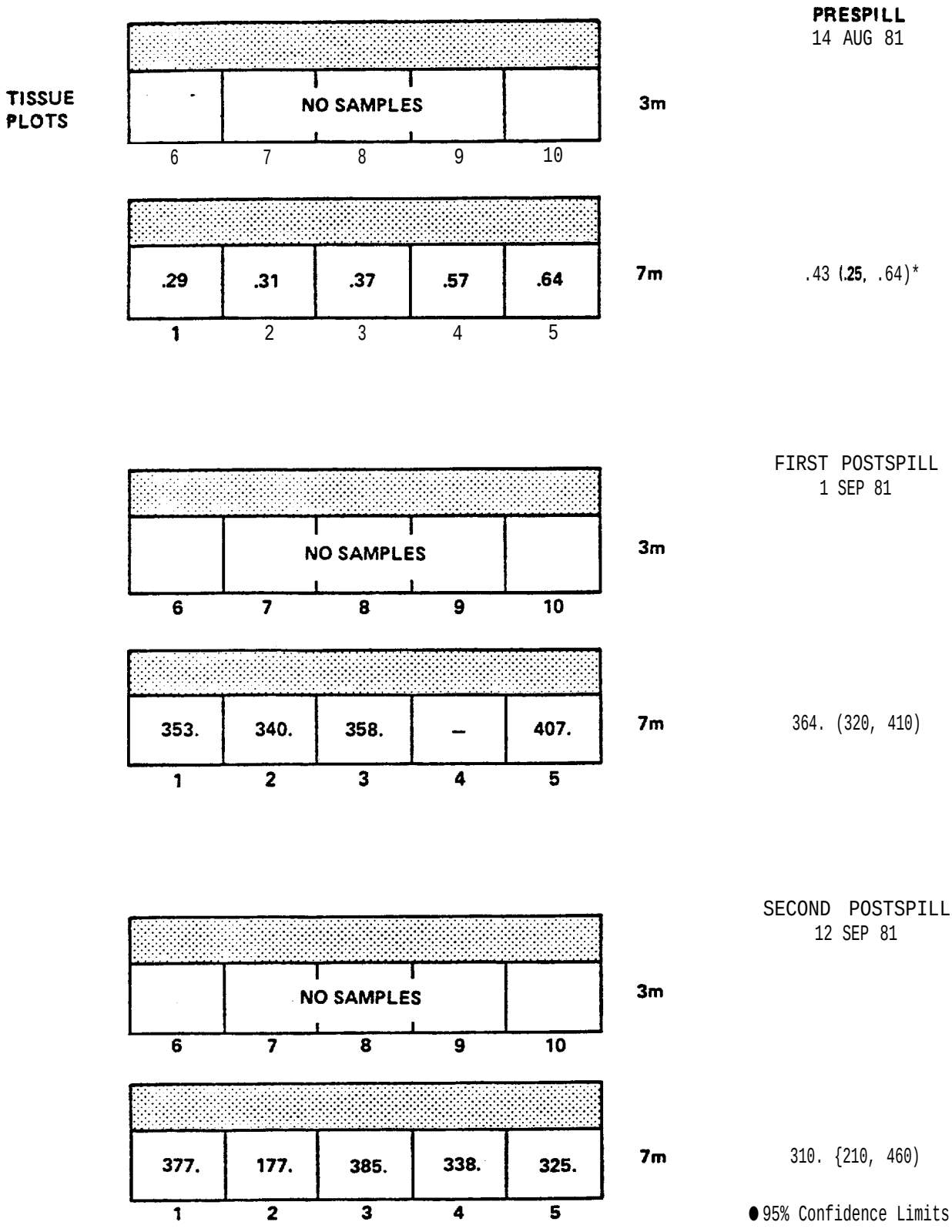

Figure 2.32. Concentrate- of oil in Astarte_borealis,_Bay 10 by UV/F (µg/g).

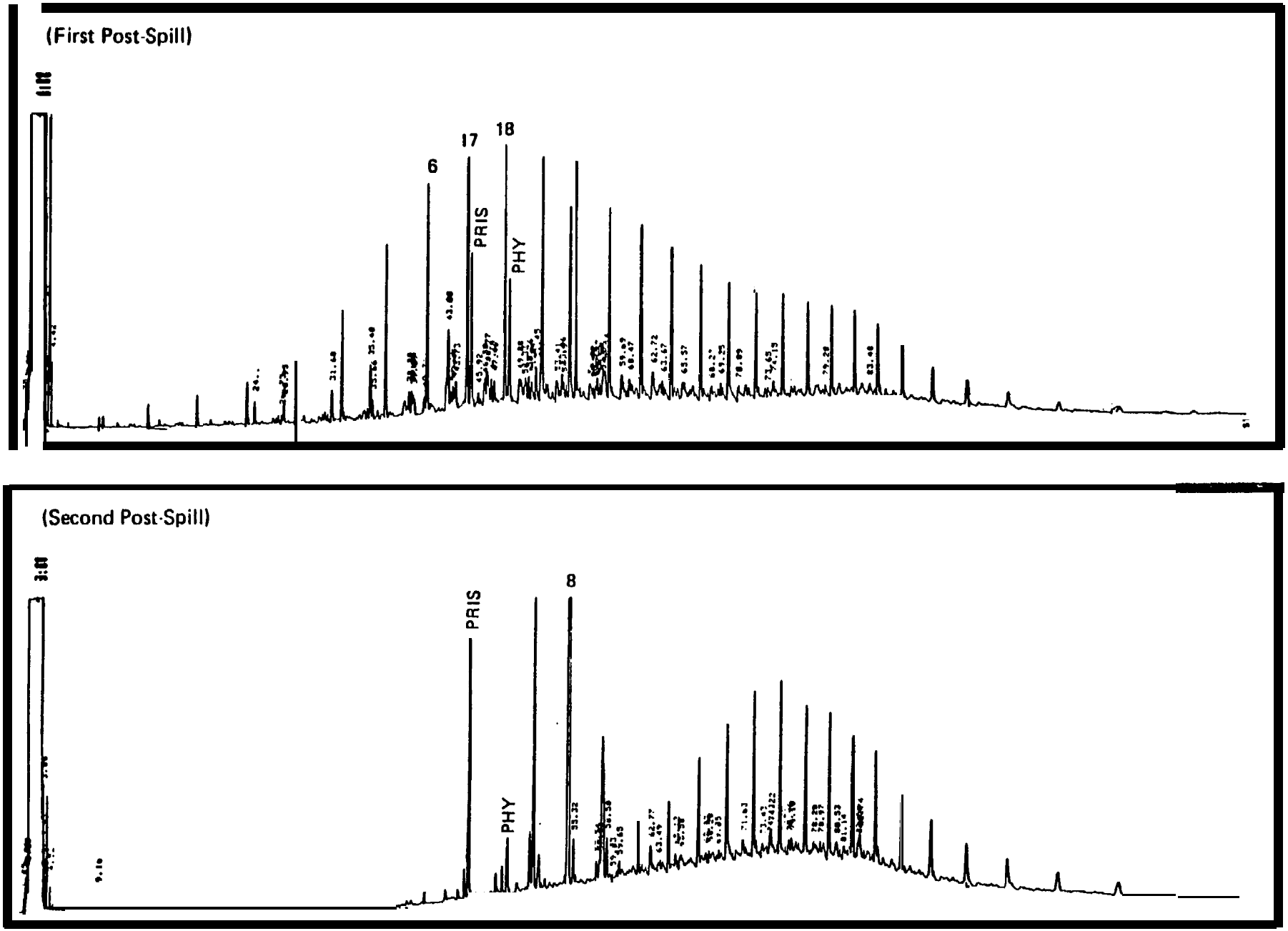

Figure 2.33. Saturated hydrocarbon GC^2 profiles of Astarte sample from Bay 9.

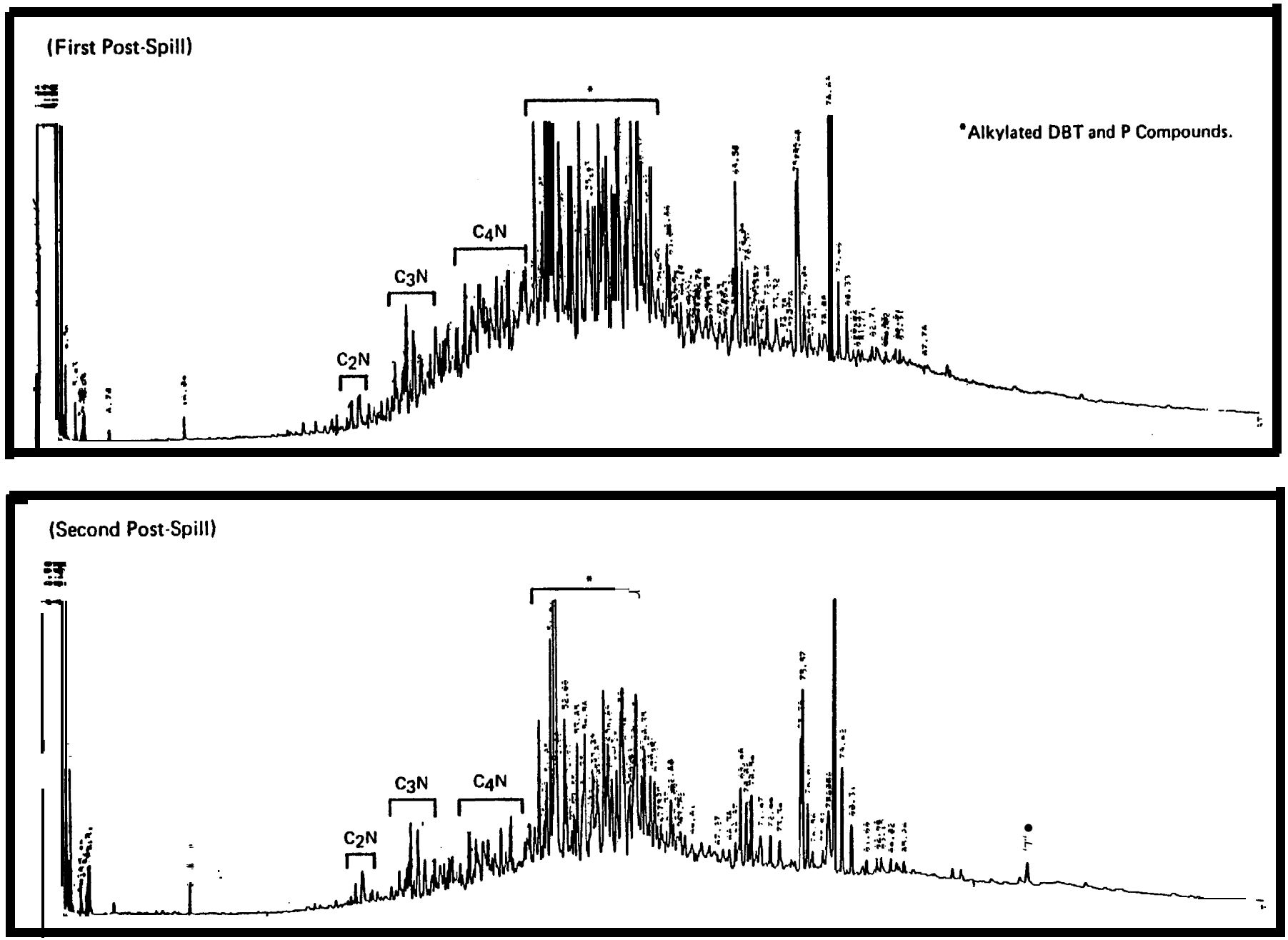

Figure 2.34. Aromatic hydrocarbon GC[2] profiles of Astarte sample composite from Bay 9.

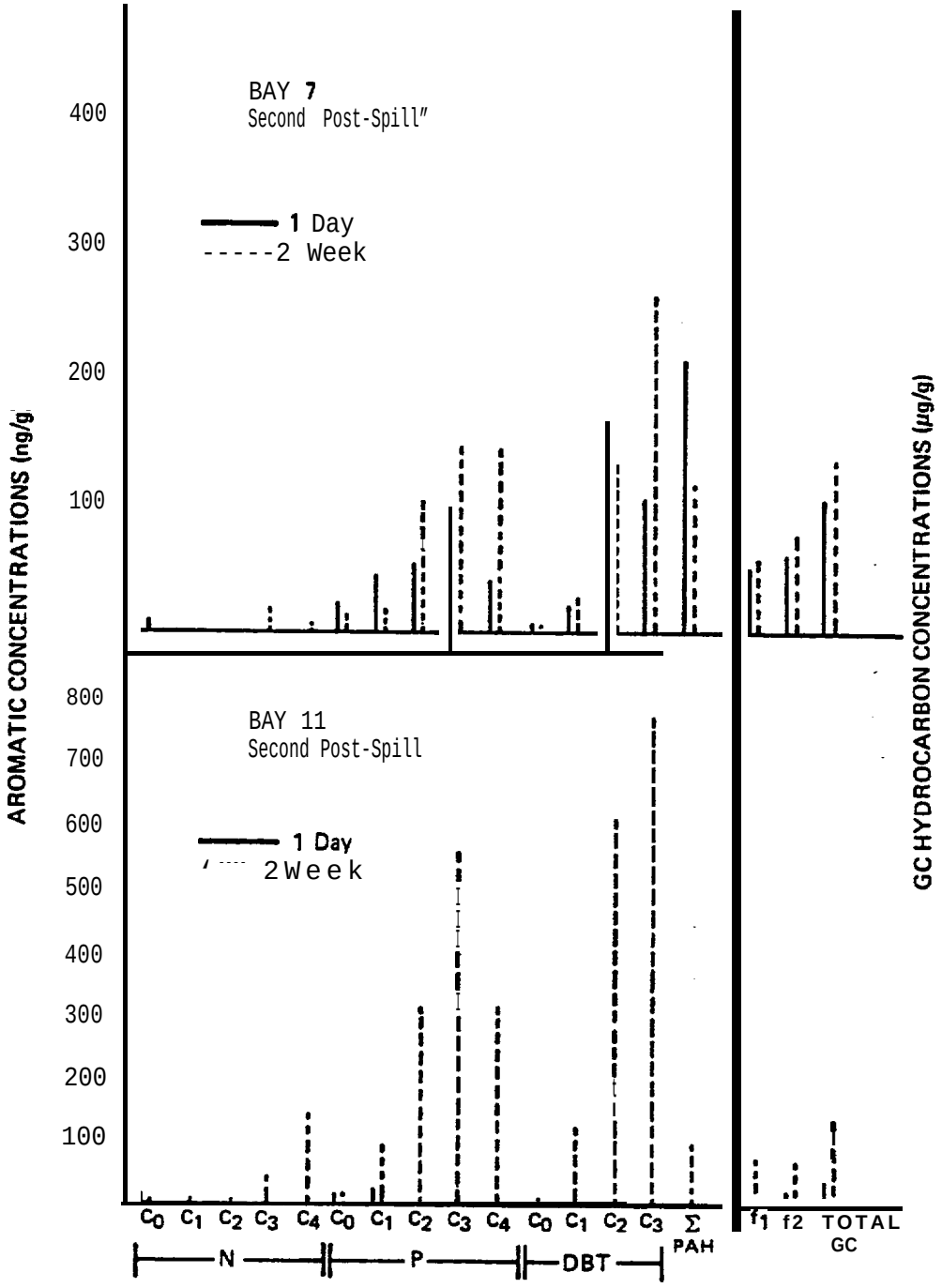

Figure 2.35. **Astarte aromatic profiles (Bays 7 and 11).**

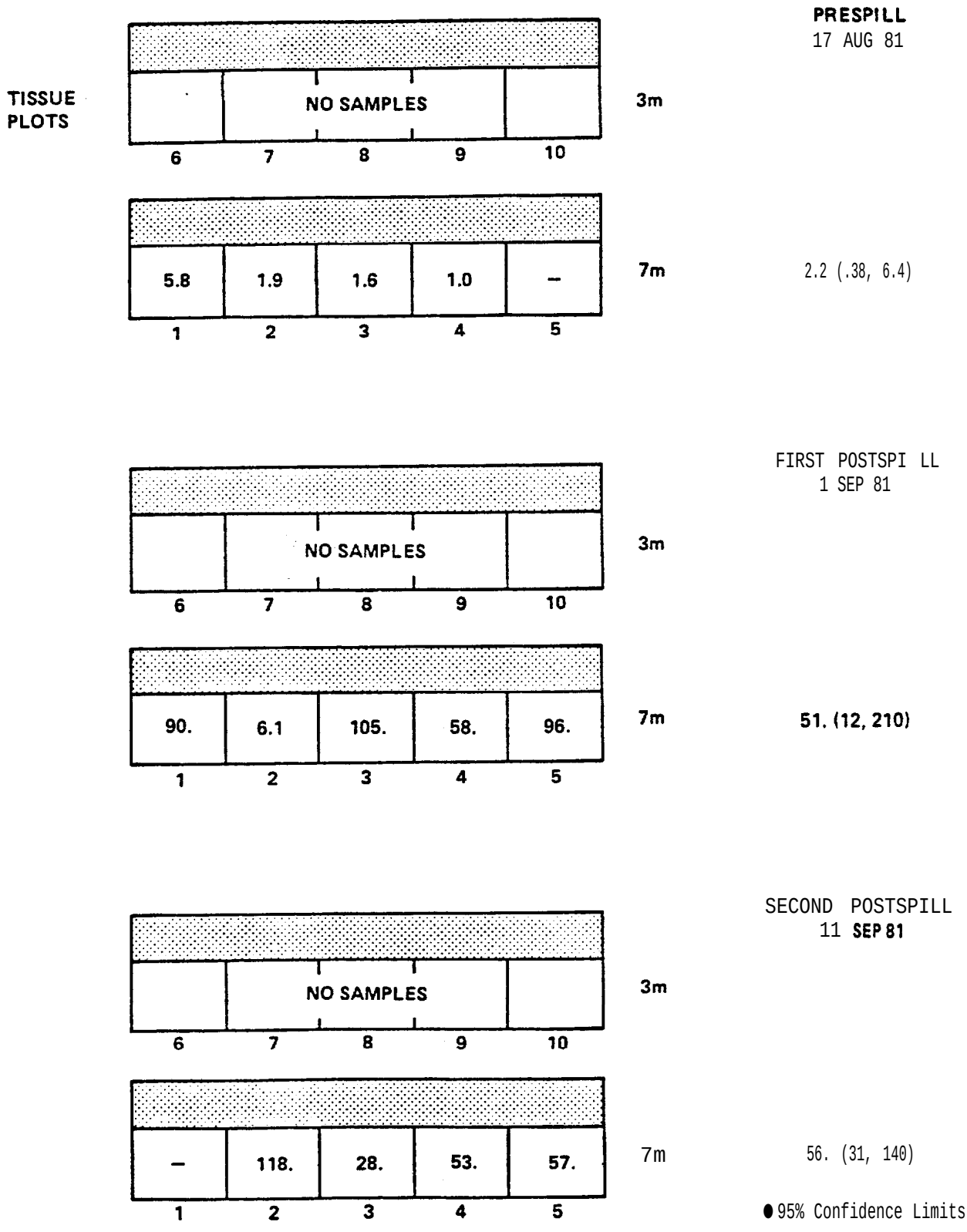

Figure 2.36. Concentrations of **oil in Astarte borealis, Bay** 7 **by UV/F (µg/g).**

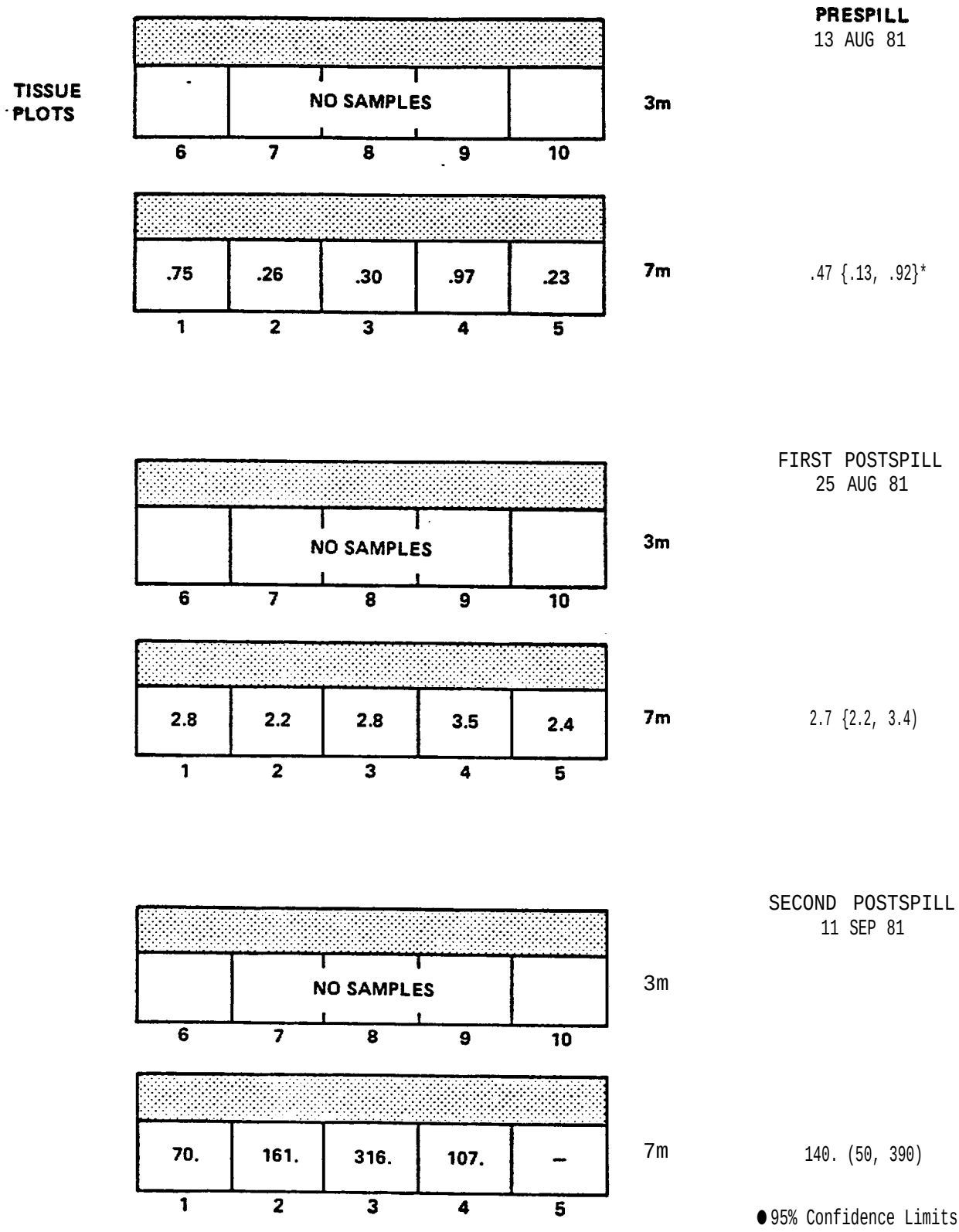

Figure 2.37. Concentrations of oil in Astarte borealis, Bay 11 by UV/F (µg/g).

Astarte, Bay 9, l-day post-spill, 7 meter

Astarte, Bay 9, 2-week post-spill, 7 meter

Serripes, Bay 10, l-day post-spill, 3 meter

Serripes, Bay 10, 2-week post-spill, 7 meter

Extracts were available in sufficient quantities for PANH analyses of these samples.

The results shown in the following GC/MS/DS data packets illustrate that PANH compounds were only detected at low levels (<10 rig/g) in the l-day post-spill Astarte sample. In this sample, dimethyl and trimethyl phenanthridines or acridines were detected. The other samples did not contain detectable PANH compounds. PANH compounds would be present at levels two orders of magnitude lower than the aromatics. The Astarte aromatic values (Figure 2.30) were the highest of any of the animals (1000-2000 rig/g). Therefore, it is entirely consistent to find the PANH values of < 10 rig/g. The detection limit for the PANH compounds was <5 rig/g.

2.2.4.1 PANH **Compounds in Oil.** The accompanying figures are the reconstructed mass spectra of the polycyclic aromatic nitrogen heterocyclic (PANH) compound fraction of the Lagomedia crude oil used in the BIOS Program. C_3-C_6-quinolines, C_2-C_5 acridines, or phenanthridines, benzacridine, and C_1-C_2 benzacridines were identified at concentrations about two orders of magnitude lower than the aromatics (See Appendix I).

2.2.4.2 **Astarte** borealis: Bay 9: 1 Day. Only trace concentrations (< 10 rig/g, parts per billion) of dimethyl- and trimethyl-acridines or phenanthridines were detected in Astarte borealis collected from Bay 9 one day after the spill (See Appendix II).

2.2.4.3 **Astarte borealis: Bay 9: 2 Weeks. No PANH at concentrations above the detection limit of 5 rig/g were detected in this sample (See Appendix I I I].**

2.2.4.4 Serripes groenlandicus: Bay 10: 1 Day. No PANH at concentrations above the detection limit of 5 rig/g were detected in this sample (See **Appendix IV).**

2.2.4.5 Serripes groenlandicus: Bay 10: 2 weeks. No PANH at concentrations above the detection limit of 5 rig/g were detected in this sample (See **Appendix V).**

2.3 Discussion

The analytical results presented here and in Boehm (1982) considerably increase our knowledge of the differential fate and behavior of chemically dispersed and surf ace oil. Furthermore, the transport of oil to the benthos, its route of transport to benthic organisms (oil acquisition), and the species-specific chemical nature of biotal oil deputation are revealed in the wealth of data obtained in this study. We will discuss some of the most important observations and trends here as they pertain to the behavior of oil in the experiments, and to specific important transport paths and biotal impacts.

The quantities of oil driven into the water column as a result of chemical dispersion are far greater than those that result from transport of untreated surface oil into the water column. Concentrations of chemically dispersed oil in the water column ranged from 1 to greater than 50 ppm (-100 ppm) during the dispersed oil discharge and for as long as twelve hours after discharge ceased at some points in Bay 9. Differential movement of oil released at different points along the diffuser resulted in direct northward movement of oil at greater depths of release (10 m) and initial southerly movement of oil at shallower depths followed by subsequent reversal of direction and "reinvasion" of Bays 9 and 10 four hours after formal oil/dispersant discharge ceased. The dispersed oil plume formed a very stable layer of oil in the water column f or perhaps 6-13 hours after dispersal. Dispersed oil droplets carried by strong shore currents were advected for considerable distances without a significant change in the composition of the oil. Whether this occurred due to the stability of the small (-10 μm) oil droplets, thus retarding f ractionat ion (i ● ., dissolution or evaporation), or whether particulate and dissolved parcels of oil traveled coherently due to strong advection (0.5 knot currents), is difficult to ascertain. Results of large volume water samplings which were taken outside of these concentrated plumes and after the passage of the highest concentrations indicated that a physical-chemical fractionation of hydrocarbon compounds did occur. It is, however, quite significant that fresh oil with its full suite of low molecular weight saturated and aromatic components persisted as a coherent plume for considerable periods of time (6-13 hours), apparently cut off from evaporative loss from either the dissolved state or by advection to the surface. Indeed, confirmation of this coherent oil layer was made by fluorescence profiling and by discrete sampling, sometimes indicating a tenfold

increase in water-borne oil concentrations within a water layer sandwiched by lower concentrations of more highly weathered oil. The persistence of low molecular weight saturates (C 6-C 10 alkanes) and alkylated benzenes and naphthalenes in the plume in similar proportion to the total petroleum in the neat oil was unexpected. Surely the subsurface release of dispersed oil accounted for this. A surface release followed by application of chemical dispersants would have allowed some loss of light aromatics to occur by evaporation.

The very striking similarity between the BIOS dispersed oil plume behavior and that observed in the Ixtoc I spill (Boehm, Barak, Fiest and Elskus 1982) is of no small importance. A subsurface release of oil that creates small oil droplets either through shear (Ixtoc) Or through stabilization through chemical dispersion (BIOS) with resulting droplets advected by strong currents, results in subsurface coherent plumes of unweathered fresh oil with a full contingent of toxic aromatics. The similarities between the two events are also striking given the 25°C water column temperature differential between Gulf of Mexico and Arctic waters. Of course these initial high levels of oil (roughly 10 ppm in the Ixtoc I and 10 ppm and greater in the BIOS scenarios) will eventually be reduced through dilution and diffusion even if the coherent subsurface plume persists as it did for 20 km or so in the Ixtoc I spill.

During and after the dispersed oil experiment, there was little evidence for either the large-scale beaching of dispersed oil or the surfacing, in the water column, of dispersed oil. However, both phenomena did occur to minor extents and resulted in some important information. Oil that was found adhering to the Bay 9 beach was present at low levels (5-10 ppm). The oil had weathered significantly, due mainly to losses of low molecular weight components. Both the concentration of oil on the beach and its composition were nearly identical to those found in the offshore bent hic sediments implying a detectable, but low sorptive affinity of dispersed oil. Oil which did appear to have coalesced at the sea surface was highly weathered through loss of low boiling saturates and aromatics. The state of weathering of this surface oil sampled several hours after initial dispersed oil discharge was equivalent to that of nine-day-old beached surface oil (Bay 11). Thus it appears that the coalesced oil formed after solubles were stripped from the oil in the water column with the coalesced oil forming from a weathered residue.

Oil did impact the sediments of Bays 9 and 10 immediately after the dispersed oil spill where initially a significant amount of the sedimented oil (-20%) resided in the surface floe. Sedimentation rates were estimated to be in the 2-10 **mg/m^2/day** range. Subsequently, the floe was transported elsewhere, probably offshore, because floe from **all** bays sampled in the **second** post-spill period (September 11) was free of any detectable oil. Levels of oil in the sediments, however, remained elevated (l-5 ppm) in Bays 9 and 10 and although this dosing is considerably less than a "massive" dosing, it will continue to affect **benthic biota** for an unknown period of time. The overall sediment impact due to passage of dispersed oil through Bays 9 and 10 was minimal, with less than **l** % of the discharged oil probably residing in the sediment at any time.

Results from the initial sampling of sediments indicated that 80 % of the oil detected in the top O-3 cm was not associated with the floe. This is in contrast to results **from other spills [e. g., Boehm, Barak, Fiest and Elskus 1982; Boehm, Wait, Fiest and Pilson 1982) and to experimental tank studies (Gearing et al., 1980] in which most of the initial l y sediment-associated oil was in the f loc layer. What appears to be occurring in the B 10S dispersed oil spill is a low level, direct and rapid penetration of dispersed oil into the bulk surface sediment, presumably a process mediated by the decrease of the oil's interracial tension due to chemical dispersion allowing for penetration of the solid interface perhaps into interstitial waters. Indeed chemical results from polychaete analyses in Bays 9 and 10 (Norstrom and Engelhardt, 1982) revealed an initial uptake of an alkylated benzene and napthalene (i. e., water-soluble fraction) enriched petroleum hydrocarbon assemblage in Bays 9 and 10 only, " perhaps associated with interstitial water penetration of fractions of the oil.**

The Bay 7 "control" did receive 50-100 ppb of dispersed oil in the first few days after the discharge. This quantity of oil was measured directly (Green et al., 1982) and was monitored indirectly through hydrocarbon body burdens in filter-feeding bivalves (i.e., M ya, **Serripes).** Direct sediment analyses and indirect evidence f rom deposit-feeding animals **(Macoma, Strongylocentrotus)** indicate, however, that oil impact to Bay 7 sediments was quite minimal with only patchy low level inputs noted. The Bay 7 analytical results point to an important conclusion regarding application of **UV/F** and **GC^2** techniques to the BIOS **study.** While background (by **UV/F)** levels of **"oil** equivalents" in the sediments was -0.5 ppm, many samples did **exhibit** post-spill oil levels of 1.0- 1.5 ppm. In this concentration, range levels were too low to unambiguously yield an oil/no oil

decision based on GC2. Oil levels of ~1.0 ppm would contain individual component concentrations (i.e., n-alkanes) of ~.01 ppm (or 10 rig/g). Due to significant biogenic background in the GC2 traces, this level of individual components was often too low to see in the GC2 traces. Thus UV/F becomes a key to assessing oil concentrations in sediments. However, in several cases in Bay 7 sediments, low UV/F levels (-0.3 ppm), generally associated with background levels, were shown by GC 2 to contain small amounts of oil. The weathering of oil while in transit to Bay 7 with resulting loss of water-soluble aromatics and a concomitant decrease in UV/F response caused whatever oil was seen in Bay 7 sediments to be relatively enriched in saturates (not detectable by UV/F). Thus the two techniques of UV/F and GC2 proved to be an extremely powerful complemental y set.

Water-borne oil in Bay 11 was initially confined to the surface (O-2 meters) layer during which time large-scale transport of oil to the benthos via sorption and sinking did not occur. Through large volume water samples, low levels (ppb) of oil were detected in mid-depth and bottom waters largel y in a particulate form, prior to any possible cross-contamination from the dispersed oil spill occurring a week later. That oil did impact the sediment in Bay 11 prior to the dispersed oil spill is evident from uptake patterns of all of the benthic animals, especially those of the deposit-feeders Macoma and Nuculana and of the filter-feeder Serripes which all revealed uptake of oil, albeit at lower levels relative to those which were acquired in the dispersed oil scenario, prior to any possible cross-contamination from Bays 9 and 10. We do know that the dispersed oil's inf luence was far-ranging including a transient water column impact at Bay 7 causing elevated levels of oil in all benthic biota, especially the filter-feeders Mya and Serripes. Thus it may be logical to "subtract" the observed Bay 7 animal levels from the Bay 11 values to derive a "pure" Bay 11 result for the second post-spill sampling. Using this logic, it can be concluded that although low levels of oil are acquired in Bay 11 by the filter-feeders, the major Bay 11 impact is on the deposit-feeders which are more closely linked to the sediments and which acquire weathered oil from off of the beach face.

The most significant findings of the study concern the relationship between water-borne levels of oil, sediment concentrations and levels in benthic biota. Initial uptake of oil by Mya and Serripes is from the water column wherein oil is acquired through pumping of contaminated seawater through gills. Most of this oil initially resides in the animal's gut as confirmed through Serripes dissections. Chemically, even the initial

oil residues in the gut and muscle tissue are different. The more water-soluble aromatics (naphthalene, alkylated benzenes) are transported to the muscle tissues (including gills) more rapidly, with the phenanthrenes and dibenzothiophenes preferentially located in the gut. During the first two weeks after the spill, however, it is these higher molecular weight aromatics which persist, the water-soluble aromatics being depurated more readily.

Initial levels of oil in filter-feeders from Bay 7 are equal or greater than those from Bays 9 and 10, where water column levels of oil were 20 to 200 times as great. Sediments are ruled out as an oil-biotal intermediary due to the near absence of oil in Bay 7 sediments. Thus one must postulate that while& and Serripes from Bays 9 and 10 either cease pumping due to water column levels or die after' initial accumulation of oil, animals in low-to-moderately contaminated waters continue to pump and acquire oil as long as it is present in the water. At water column concentrations of 50 µg/l (50 ppb), a clam (1 g dry weight) pumping at a rate of 1 liter per hour would pass 1.2 mg of oil through its body in 24 hours, more than enough to acquire a 100-500 ppm concentration. As levels of oil in Bays 9 and 10 were much higher, 1-50 ppm initially and 100-200 ppb for at least a day to a day and a half after cessation of the oil spillage, opportunities for greater bioaccumulation in Bays 9 and 10 were available but were probably not achieved due to either saturation in the gut, inability to transport oil across the membranes fast enough to acquire more oil, or a wholesale cessation of pumping. The latter explanation is the most likely.

Mya truncata and Serripes groenlandicus are filter-feeders and accumulate oil primarily from the water column. They depurate 60-75 percent of the accumulated oil wit hin two weeks, even though the sediments in which they reside remain contaminated with oil. On the other hand, Macoma calcarea and Nuculana minuta are deposit-f ceders, and accumulate petroleum hydrocarbons primarily from the sediments. In controlled laboratory experiments, Roesijadi et al. (1978) showed that the deposit-feeder, Macoma inquinata, accumulated higher concentrations of aromatic hydrocarbons from Prudhoe Bay crude oil-contaminated sediments than did the filter-f ceder, Protot haca staminea. In the BIOS study, the deposit-feeders continued to accumulate hydrocarbons during the two weeks after the spill (Bays 9 and 11) or became heavily contaminated immediately after the spill and retained the hydrocarbons for at least two weeks (Bay 7 and 10). The GC[2]

profiles of tissue extracts of the deposit-feeders show evidence of uptake of oil from sediment, rather than from the water column, after an initial rapid uptake of perhaps 30-50 ppm oil from the water column.

As discussed previously, the two oil spill experiments conducted introduced oil into the nearshore system in two distinct manners. The Bay 11 surface oil (untreated) spill resulted in detectable water-borne oil concentrations only in the top meter or so of the water column (Green et al., 1982). That low levels of water-soluble oil may have penetrated to the benthos during the first day or so following the spill can not be confirmed from direct chemical evidence of water samples, but may have occurred, causing the low intitial increases in petroleum hydrocarbon levels and levels of water soluble aromatics in some of the filter-f ceders (M ya, Serripes, Ast arte). That oil did impact the bent hos of Bay 11 as soon as one day after the spill is indicated by the uptake of oil by Macoma, Pectinaria and Strongylocentrotus in the immediate post-spill period. Subsequent benthic impact of oil in Bay 11 is clearly indicated in increased sediment concentrations (-5 ppm) as well as by the increased uptake of oil by the deposit and detrital f ceders. The oil reaching the benthos during the 1 day to 2 week post-spill period was weathered due to evaporation/dissolution as evidenced by the loss of alkylated benzene and naphthalene compounds relative to the spilled oil.

- The uptake and deputation curves during the first several days are difficult to reconstruct due to differences in sampling times. For example, it is not clear whether higher levels of oil in Serripes in Bay 10 versus Bay 9 were due to a combination of animal behavior and water column concentration or due to the additional day during which they acquired oil. Alternative y, filter-feeders may very well have "shut down" their pumping systems in Bay 9 (or were narcotized or killed outright) due to high water column oil concentrations, while those animals in Bay 10 may have continued to pump and acquire more oil. Indeed this seems to have been the case in Bay 7. Low levels of oil (50-100 ppb) were detected in Bay 7 two days after the spill (Green et al., 1982), as were these same levels in Bays 9, 10, and at other Ragged Channel locations. Bay 7 Serripes were especially efficient at concentrating oil from these lower water column levels, with oil residing primarily in the gut initially. Serripes and Mya from Bay 7 probably did not detect those lower levels of oil and may have continued their normal pumping of water throughout the first several days after the spill.

As alike as Mya and Serripes behave vis-a-vis routes of oil uptake, they differ in the compositional nature of the oil which they retain. During the two week post-spill period of deputation, an in vivo biodegradation, presumably by a microbial population within the animal's guts, occurred to a significant extent. At this point, the similarity between Mya and Serripes erodes, because although on a gross level both species depurated oil, on a detailed chemical basis Serripes preferentially retained a high molecular weight saturated hydrocarbon assemblage as well as the higher alkylated naphthalene, phenanthrene and dibenzothiophene compounds. Mya, on the other hand, depurated all hydrocarbon components, although the water-soluble alkyl benzenes and napht halenes were depurated somewhat faster.

Thus, as the exposure levels in the water column decreased, levels of total hydrocarbons in Mya and Serripes decreased. This, plus the fact that whole, undegraded oil resided in Bay 11, 9, and 10 sediments without a concomitant increase in concentrations of oil in the filter-feeders provides evidence of decoupling of sedimentary sources of hydrocarbons from these animals. This decoupling is accented by the fact that while oil residues in sediments were not degraded, residues in the animals were microbian y degraded.

Macoma, Nuculana, Strongylocentrotus, and Pectinaria clearly are influenced by sediment oil levels more than those in the water column. Though there is some indication that low levels of soluble aromatics in the water were reflected in early oil compositions in the deposit-f ceders, steady uptake of sediment-bound oil by this group dominates. Thus, the lack of detectable sediment-bound oil in Bay 7 is reflected in much lower petroleum body burdens in deposit-feeders from this bay. Additionally, over two weeks we see much less of an indication of microbial degradation in the Bay 9, 10 and 11 deposit-feeding animals due to the acquisition of undegraded oil from the sediments appearing as a constant compositional overprint. Furthermore, those aromatic hydrocarbon components longest-lived in the sediments (i.e., alkylated dibenzothiophene and phenant hrene compounds) steadily increase in the deposit-feeders.

Thus, the various filter-feeders and deposit/detrital feeders reflect the fate of oil in the system quite well. The fact that the polychaete acquires whole oil, dominated somewhat by a water-soluble grouping of alkylated benzenes and naphthalenes, may reflect the association of oil with interstitial waters in the upper sediment column.

A similar differential behavior of filter-feeding versus detrital feeding bivalves was reported recently in an actual spill (Boehm et al. 1982b). In this study, the authors found that the benthic-dwelling Macoma balthica was slower to initially acquire oil than was the filter-feeder Mytilus edulis which resided in the phytal zone. After beaching and erosional transport, and/or direct sedimentation of oil, the petroleum body burden increased in Macoma and only slowly decreased as the sediment levels dropped. Mytilus, On the other hand, exposed to a massive initial amount of water-borne oil, depurated rapidly and almost completely over one year's time.

During the first two to three weeks after the spills, there was a notable lack of significant biodegradation of oil in the water column and in the sediments. There is no chemical evidence for the existence of biodegradation as a removal mechanism with the short-term post-spill period (3 weeks) either in the water column or in the sediment. One would have predicted higher rates of biodegradation in surface sediments, especially in the surface floe, but none was observed through degradation of the "easily" degraded n-alkanes. However, degradation of n-alkanes in the oil resulting in the classic loss of n-alkane relative to isoprenoid and other highly branched alkanes is observed within Mya and Serripes and to lesser extents in other benthic species. Rapid degradation of alkanes only occurs in vivo. Whether or not this unique finding can be ascribed to microbiotal populations within the organism itself, a likely mechanism, must be confirmed independently. We suspect that given an unspecified amount of time, microbial populations will begin to utilize the hydrocarbons as an energy source (i.e., biodegradation will become more significant).

The use of a variety of biological monitors or sentinel organisms in the BIOS study has served to delineate oil transport paths and changing environmental compartment levels with time during the immediate post-spill (O-3 weeks) period. Furthermore, this study has shown that although similarly behaving animals (e.g., Mya/Serripes; Macoma/Strongylocentrotus) may on a gross level appear to act in concert, the details of in vivo modifications and retentions of individual petroleum components are quite different and may be intimately associated with long-term biological effects on the individual benthic species.

3. HISTOPATHOLOGY

3.1 Materials and Methods

3.1.1. **Collections.** The first series of specimens of *Mya truncata* were collected by divers between August 7 and August 17, 1981. A second group of specimens was collected between August 21 and September 3, 1981, following the application of dispersed oil to Bay 11 on August 19, and of dispersed oil to Bay 9 (and subsequently to Bay 10) on August 27. Because of the unlikely possibility that any major pathological conditions caused by the oil or dispersed oil would be apparent within the approximately two-week period following the spill, a third series of samples was not collected until a year later, on August 27and 28, 1982.

Specimens of *Macoma calcarea* were collected prior to the spill only from Bay 9. Collections were made in Bay 7, the control bay, on September 2 and 3, 1981, a few days after oil and dispersant were added to Bay 9, and almost two weeks after oil was applied to Bay 11.

The dates and sites of collection and the number of specimens of each species collected for histopathology investigations are shown in Tables 3.1 and 3.2.

3.1.2. **Processing.** Specimens were fixed at the Baffin Island location by the collectors. Fixation for the 1981 collections was in Carson's modified Millonig's phosphate-buffered formalin. This fixative was used rather than the originally proposed Helly's fixative because of its ease of handling by the divers under field conditions. Neutral buffered 10 percent formalin was used for the 1982 collections for the same reason.

For fixation, larger specimens such as *Mya truncata* were to have one valve removed before being placed in the fixative. Smaller specimens such as *Macoma calcarea* were to be treated similarly if possible, or at least to have the shell cracked slightly to permit entry of the fixative. In fact, some specimens were placed intact in the fixative. The specimens were placed in fixative in small plastic tissue bags, in which were also placed coded identification tags. The bags were then sealed by having the tags rolled down and secured with attached plastic strips. The bags were packed in shipping containers and shipped to the laboratory in Duxbury, Massachusetts for histopathological analysis.

Table 3.1. Dates of collection and collection sites for specimens of *Mya truncata* for BIOS histopathology investigation

	Date collected	Bay Number	No. of Specimens
Immediate Pre-Spill	8/7-8/9/1981	9	94
	8/12/81	**11**	**63**
	8/1 4-8/ 15/81	10	84
	8/17/81	7	40
Immediate Post-Spill	8/21/81	11	59
	8/28-8/29/81	9	80
	8/29-8/30/81	10	102
	8/31/81	7	47
1 year Post-Spill	8/27/82	11	77
	8/27-8/28/82	10	73
	8/28/82	**9**	**75**
	8/28/82	7	75

Table 3.2. **Dates of collection and collection sites for specimens of Macoma calcarea for BIOS histopathology investigation**

	Date collected	Bay Number	No. of Specimens
Immediate Pre-Spill	8/9/81	9	83
Immediate Post-Spill	9/2-9/3/81	7	72
1 Year Post-Spill	8/27/82	10	83
	8/27/82	11	120
	8/28/82	7	86
	8/28/82	9	75

Fixed specimens from the 1981 collections were received at Battelle New England Marine Research Laboratory on November 6, 1981. Specimens from the 1982 collections were received on September 22, 1982.

Upon receipt at BNEMRL, the samples were removed from the shipping containers and logged in according to the coded label by station number, species, and date collected. The specimens were then washed in running tapwater for several hours and transferred to 70% ethyl alcohol until histological processing.

For processing, the specimens were trimmed to provide cross-sectional pieces of tissue which were dehydrated and embedded in Paraplast Plus.

The embedded tissues were sectioned at 5 to 6 μm and stained with hematoxylin and eosin using standard procedures. The stained sections were examined for any pathological conditions.

3.2 Results

Results of histopathological observations of tissues of Baffin Island molluscs are summarized in Tables 3.3 through 3.7. Tables 3.3, 3.4, and 3.5 show the results of observations of Mya truncata from the pre-spill, immediate post-spill, and one year post-spill collections, respectively. Tables 3.6 and 3.7 summarize the results of the pre-spill and one year post-spill observations of Macoma calcarea.

Despite indications of poor fixation, a number of pathological conditions were noted primarily in tissues sampled after the spill. The most serious of these included hematopoietic neoplasms, or blood tumors, in both species of clams studied.

Details of the pathology of each of the two species studied are provided below.

3.2.1 **Mya truncata.** The most common pathological problems observed were hemocytic infiltration, or inflammation, and the occurrence of an unidentified trematode parasite (Table 3.3, 3.4, and 3.5). Immediately following the spill, the incidence of necrotic tissue, particularly in the gills and digestive tract, increased in Bays 7, 9, and 10 (Table 3.4), but a year later this incidence had decreased considerably (Table 3.5). Necrotic lesions in the digestive tract were accompanied by an increase in the number of mucus-producing cells in the gastrointestinal tissues, and in Bay 10 by unidentified basophilic inclusions in the digestive gland tubules. Bays 9 and 10 produced a few one-year post-spill clams with granulocytomas throughout the tissues (Figure 3.1).

Table 3.3. Summary of histopathological observations of tissues of the truncate soft-shelled clam Mya truncata from the Baffin Island oil spill area prior to the application of oil and dispersant

			Condition								
Bay No.	Station	Numbers of Specimens	Hemocytosis	Necrosis	Abscesses	Digestive Tubule Vacuolization	Metaplasia	Hyperplasia	Neoplasia	Parasites	Other
	1	7	2							6	
	2	8	1					1		4	
	3	8	1					1		4	
	4	6									
	5	9								1	
9	1	21	1							2	
	2	6									
	3	12								5	
	4	10						1			
	5	9								3	
	6	9								4	
	7	9								6	
	8	9								7	
	9	9									
10	1	10								1	
	2	10								2	
	3	9								3	
	4	8								1	
	5	6	1							1	
	6	8	1							3	
	7	9	1							4	
	8	9								3	1-mass of hypertrophic hemocytes in stomach
	9	7								2	
	10	8								4	
11	1	7									
	2	9									
	3	10	1							5	
	4	9	1		1					3	
	5	11	3								

Table 3.4. Summary of histopathological observations of tissues of the truncate soft-shelled clam Mya truncata from the Baffin Island oil spill area immediately following the application of oil and dispersant

Bay	Station No.	Numbers of Specimens	Digestive Tubule condition: Hemocytosis	Necrosis	Abscesses	Vacuolization	Metaplasia	Hyperplasia	Neoplasia	Parasites	Other
7	1	10		1						3	2-mucus cells in gastrointestinal epitheliums
	2	8									
	3	10									
	4	10							1		
	5	9									
9	3	11								5	
	4	11	3							4	
	5	10	1							2	
	6	8								4	1-fibrous connective tissue
	7	11								3	
	8	10		2	2					2	
	9	9		1						3	
	10	10		1						6	
10	1	11		5						4	
	2	10	1							4	
	3	11		4						2	3-mucus cells in gastrointestinal epitheliums
	4	10		3						1	
	5	9	2	1						2	
	6	10	1							5	
	7	9	1		1					4	
	8	11					1			5	1-basophilic inclusions in digestive mass of hypertrophic hemocyte tubules
	9	11	1							7	1-basophilic inclusions in digestive tubles
	10	10								2	2-basophilic inclusion in digestive tubules
11	1	12								5	
	2	11	1							5	
	3	13								6	
	4	13								5	
	5	10	1							1	

Table 3.5. Summary of histopathological observations of tissues of the truncate soft-shelled clam Mya truncata from the Baffin Island oil spill area one year following the application of oil and dispersant

			Condition							
Bay No.	Station	Numbers of Specimens	Hemocytosis	Necrosis	Abscesses	Digestive Tubule Vacuolization	Metaplasia	Hyperplasia	Neoplasia	Parasites	other
7	1	15	1	1						8	
	2	15	3							3	
	3	15	4							5	
	4	15	2							5	
	5	15	6	1	1					1	
9	1	15								6	1-granulocytomas
	2	15	2	1			1			7	
	3	15	3	1						6	1-granulocytomas
	4	15	2							6	1-fibrous connective tissue
	5	15	2							5	
10	1	15	1							7	1-granulocytomas
	2	15	2							4	
	3	15								4	1-granulocytomas
	4	15	2	1						5	
	5	15								3	1-inclusions in digestive tubule epitheliums
11	1	15								9	
	2	15								8	
	3	15	1	3						7	
	4	15	1						1	7	1-cysts on gill from gregarine-like organism
	5	17							2	10	

Table 3.6. Summary of histopathological observations of tissues of the chalky macoma Macoma calcarea from the Baffin Island oil spill area prior to and immediately following the application of oil and dispersant

Bay	No.	Station	Numbers of Specimens	Hemocytosis	Necrosis	Abscesses	Digestive Tubule Vacuolization	Metaplasia	Hyperplasia	Neoplasia	Parasites	Other
							condition					
9	1		11									
	2		9									
	3		20									1-unidentified inclusions in testis
	4		28	1	1	2					1	
	5		15									
7	1		15		1	1						1-granulocytomas
	2		16		1						2	
	3		23		3		1				2	1-small cyst in digestive tubule
	4		7		1							
	5		11		3							

Table 3.7. Summary of histopathological observations of tissues of the chalky macoma Macoma calcarea from the Baffin Island oil spill area one year following the application of oil and dispersant

Bay No.	Station	Numbers of Specimens	Condition								
			Hemocytosis	Necrosis	Abscesses	Digestive Tubule Vacuolization	Metaplasia	Hyperplasia	Neoplasia	Parasites	Other
7	1	16									l-unidentified inclusions in digestive gland
	2	15								1	
	3	20								1	
	4	18								1	
	5	17		1	1					3	l-encysted inclusion
9	1	13									
	2	15		2						1	
	3	15								1	
	4	16		2							
	5	16		3				1			
10	1	18			1	1				2	
	2	20	1	1		3				1	
	3	16	2	2	1	1					
	4	14									
	5	15		1	1	3				1	
11	1	25	1	3	1	13				5	l-encysted inclusion
	2	31		1	1	22			1	7	
	3	26	3		1	17	1			7	
	4	13	1			8				4	
	5	25	1		1	3				1	l-unidentified inclusions in gonad

Figure 3.1. Granulocytoma in testis of truncate soft-shelled clam, Mya truncata, from Bay 9 one year following oil spill.

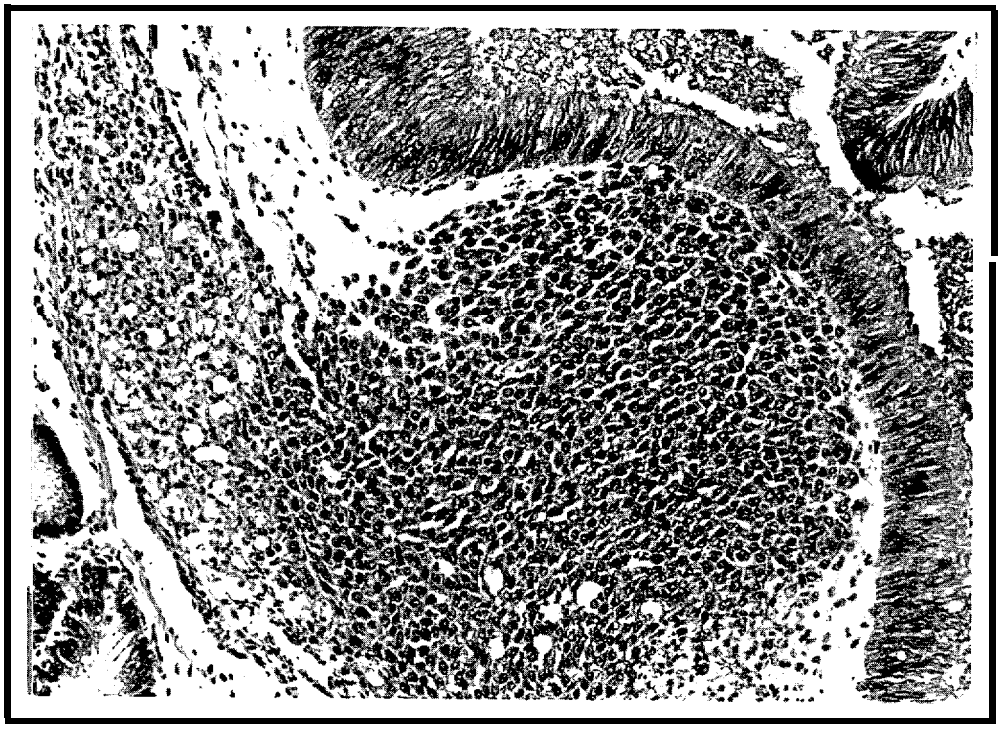

Figure 3.2. **Neoplastic hemocytes in tissues of the truncate soft-shelled clam,** M ya **truncata,** collected from **Bay 7 immediately following** the oil spill.

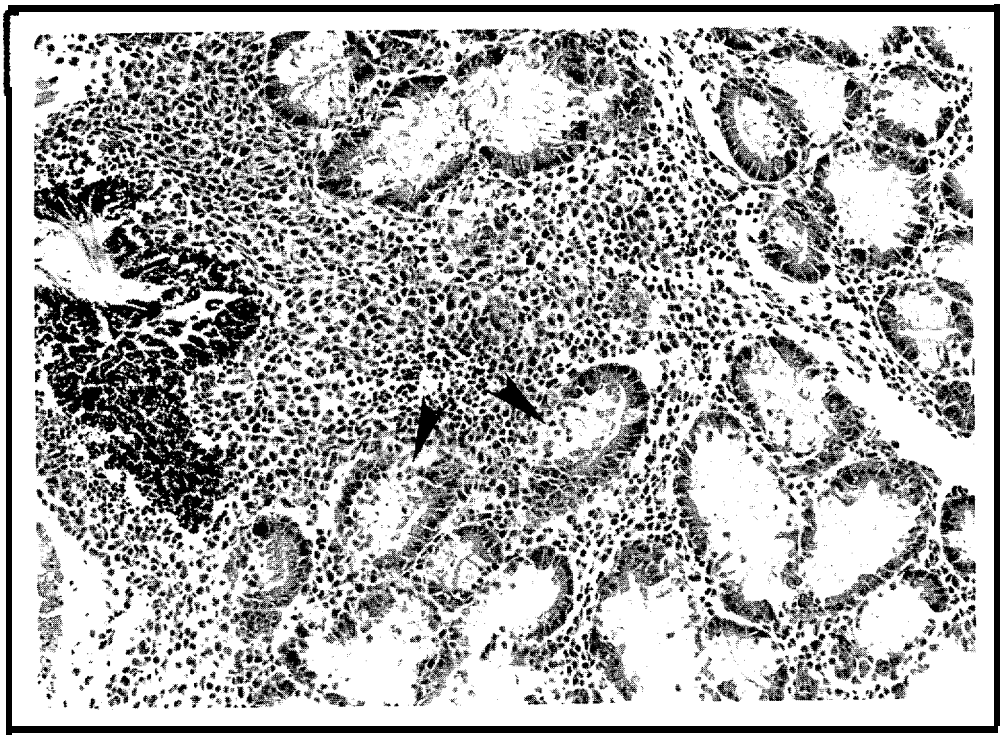

Figure 3.3. Hematopoietic neoplasm in digestive tubule area of truncate soft-shelled clam, Mya truncata, collected from Bay 11 one year after oil spill. Note invasion of digestive tubules by neoplastic cells (arrows).

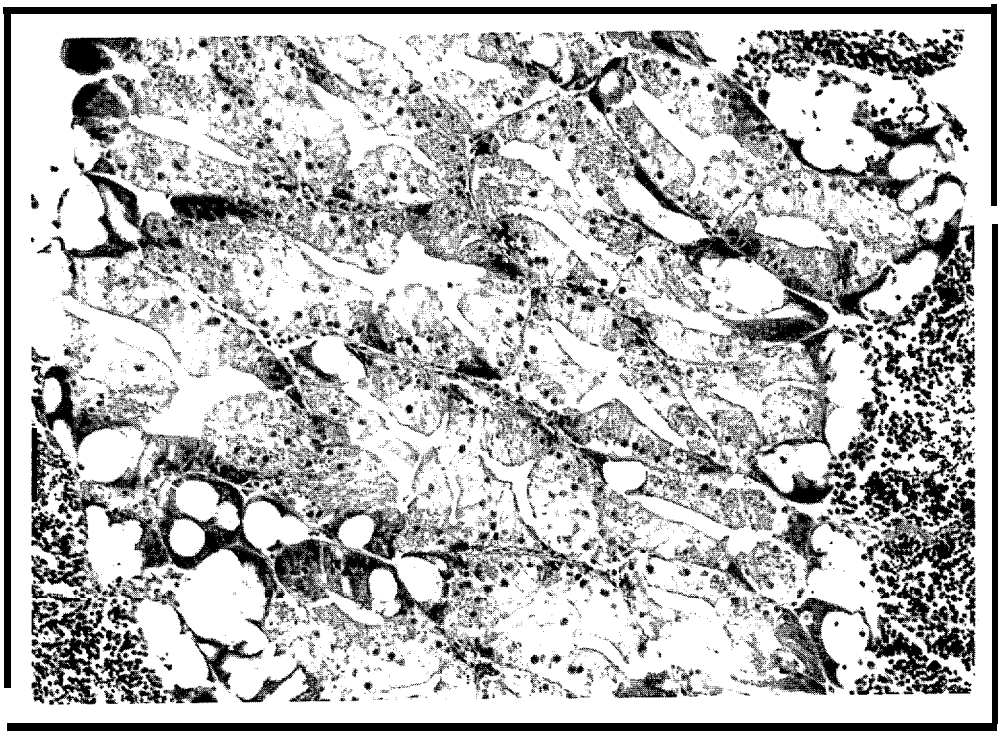

Figure 3.4. Normal digestive tubules of the chalky Macoma, Macoma calcarea, from Bay 11 one year following the oil spill.

Figure 3.5. Excessively vacuolated digestive tubules of the chalky Macoma, Macoma calcarea, from Bay 11 one year following the oil spill.

by the oil has not been established. Of the five observed incidence of neoplasms, four occurred in Bay 11 a year after the application of the oil.

The presence of granulocytomas in several specimens of Mya truncata from Bays 9 and 10 after a year also is of interest. Lowe and Moore (1979) suggest a relationship between this non-neoplastic inflammatory cellular condition, which they describe in the marine mussel Mytilus edulis, and water quality. They point out that mussels from areas of chronic domestic and industrial pollution have a high incidence of granulocytomas, whereas mussels exposed to low-level pollution exhibit a low to zero incidence of the condition.

The parasite burden in Bay 11 also appears to be higher than in the other bays. Both species, but especially Mya truncata, were quite heavily parasitized before and after the oil spill. This degree of parasitism might have an effect on the ability of the clams to withstand toxic effects of the oil. Conversely, the effects of the oil could lead to an increased parasite burden.

A small amount of vacuolization of digestive tubule epithelium is not uncommon and may be normal. The degree to which the tubules of M. calcarea from Bay 11 a year after the oil spills were vacuolated seems excessive when compared to the condition of the tubules from M. calcarea specimens at other times and at other sites. It is undoubtedly related to diet or feeding, but whether there is an effect of the oil is not fully understood at this time. Similar conditions of the digestive tubule epitheliums were reported in bivalve molluscs contaminated by the Amoco Cadiz oil spill (Wolfe et al., 19s1; Neff and Haensly, 1982).

None of the pathological effects noted can be attributed directly to the oil, although there are indications of some relationship between the experimental oil spill and the noted effects. More needs to be known, not only about the relationship of oil and dispersed oil to the observed lesions, but about how the various toxicants affect a mollusc's ability to mobilize its own natural defense mechanisms.

4. BIOCHEMICAL EFFECTS OF OIL

The objective of the biochemistry program was to compare the state of health of infaunal bivalve molluscs from bays at the BIOS experimental site receiving dispersed oil, undispersed oil, and no oil. A suite of diagnostic biochemical tests of proven utility was used to diagnose sublethal pollutant stress in four populations of molluscs from the oil spill site.

4.1 Materials and Methods

There were four experimental bays used in these experiments, located on the northwest coast of Baffin Island, Northwest Territories, Canada. Bay 7 was considered a reference bay (though it received oil), Bays 9 and 10 received dispersed oil, and Bay 11 received oil alone. A Lagomedio crude oil and the dispersant, Corexit 9527, were used in these field experiments.

Mollusc specimens were collected by divers, using an air-lift system, at ten stations located along two transects paralleling the shore at the 3-meter and 7-meter isobaths in each bay. Stations 1-5 were along the 7-meter transect and Stations 6-10 were along the 3-meter transect. Specimens were collected several days before the spill, 1-4 days after the spill, and approximately 2 weeks after the spill. Some samples were kept over night or longer before freezing.

Mollusc specimens for biochemistry were frozen and returned to the U.S. on dry ice. A complete set of the truncate soft-shell clam Mya truncata was available for analysis. Only small numbers of Macoma calcarea, Astarte borealis, and Serripes groenlandicus were available from a few bays at each sampling time. Therefore, we examined only Mya, but analyzed more replicate samples of this species from each collection than originally proposed. A total of 228 specimens of Mya truncata were analyzed for carbohydrates and lipids and 230 specimens were analyzed for tissue free amino acids.

In the laboratory, individual clams were thawed, shucked, and weighed. Tissue glucose, glycogen, and other glucose-containing carbohydrates (mainly trehalose, a glucose disaccharide) were analyzed with the Beckman automatic glucose analyzer after

selective hydrolysis according to the method of Carr and Nef f (1983). The tissue was homogenized. An aliquot of the homogenate was centrifuged and glucose concentration in the supernatant was measured. Another aliquot of the homogenate was incubated overnight in acid buffer with amyloglucosidase which selectively and quantitatively hydrolyzes glycogen to glucose. The homogenate was centrifuged and the glucose concentration in the supernatant was measured. For the other carbohydrates, an aliquot of the tissue homogenate was incubated in concentrated HCL for three hours at 100°C. The mixture then was neutralized with 12N NaOH and centrifuged. Glucose concentration in the supernatant was measured. Glycogen concentration was calculated as glucose concentration in the am yloglucosidase digest minus glucose concentration in the original supernatant. The concentration of other glucose-containing carbohydrates was calculated as the glucose concentration in the acid digest minus the glucose concentration in the amyloglucosidase digest.

Total lipids were determined according to the methods of Holland and Gabbott (1971). An aliquot of the tissue homogenate was extracted with chloroform-methanol, centrifuged, and the supernatant dried and taken up in chloroform. Total lipids were measured spectrophotometrically following treatment with H2s04 at 200°C.

Whole soft tissues of clams were extracted and analyzed fortissue free amino acids by methods similar to those described by Roesijadi and Anderson (1979). The tissues were homogenized in7 percent trichloroacetic acid. The homogenate was centrifuged and thesupernatant was washed three times with diethyl ether to remove trichloroacetic acid. The supernatant then was lyophylized and dissolved in 0.1N HCl. Samples were analyzed with a Waters Associates gradient high-performance liquid chromatography equipped for post-column derivatization with O-phthalaldeh yde and f luorescence detection.

Data were analyzed for statistically significant differences between control and treatment means by the Mann-Whitney one-tailed U-test, Student's t-test and Kruskal-Wallis one-way analysis of variance. The Spearman rank correlation test was used to detect association between pairs of biochemical parameters among animals from cliff erent sampling times and treatment groups.

4.2 Results

Based on analyses of petroleum hydrocarbons in tissues of five species of molluscs, performed as part of this program (see Section 2), all four bays received some oil during or after the simulated oil spill. Mya truncata from Bay 10 became the most heavily contaminated with petroleum hydrocarbons, followed in order by clams from Bays 9, 7, and 11 (Table 4.1). Mya from the two dispersed oil bays (Nos. 9 and 10) accumulated the most oil immediately after the spill. The mean concentration of petroleum hydrocarbons in clams from Bay 11 (oil alone) increased between one-day and 2 weeks post-spill. Clams from the reference bay (Bay 7) were contaminated with a mean of 114 ppm (range 60-194 ppm in clams from the 5 stations on the 7-meter transect) one day after the spill, indicating that some oil reached this bay. Before the spill, clams from all four bays contained similar low levels of petroleum hydrocarbons.

There was a great deal of variation among replicates, experimental bays, and sampling times in the concentration of carbohydrates and lipids in the tissues of Mya truncata (Table 4. 1). Mean concentrations of free glucose were higher in clams from all four bays two weeks after the simulated oil spills (second post-spill sample) than in samples collected at the two earlier sampling times. There was a drop in the concentration of free glucose in clam tissues between the pre-spill and first post-spill samples in Bay 10 (dispersed oil) and 11 (oil alone). Glucose concentrations in tissues of clams from the four bays were nearly the same at the time of the second post-spill sampling. In clams collected before the spills, clams from Bays 9 and 10 had significantly lower tissue glucose concentrations than clams from Bay 11. Immediately after the spill, clams from the three experimental bays (9, 10, and 11) had significantly lower tissue mean glucose concentrations than clams from the reference bay (7). Clams from the more heavily oiled of the two bays receiving dispersed oil (Bay 10) had a significantly lower tissue glucose concentration than clams from the less heavily oiled, dispersed oil bay (Bay 9).

There was a tendency for tissue glycogen concentration in clams to increase between the first, second, and third collections, particularly in clams from the reference bay. In clams from Bays 10 and 11, mean tissue glycogen concentrations dropped between the first and second post-spill samples. The mean concentration of tissue glycogen in

Table 4.1. Carbohydrates and lipids in tissues of the truncated soft-shelf clam Mya truncata collected from the BIOS site before and after the simulated oil spill. All values are in mg/g wet tissue. Mean concentrations of petroleum hydrocarbons in tissues of the clams also are given.

Station/Collection	Petroleum (ppm)	(mg/g wet wt. + SE) Glucose	Glycogen	Other Carbohydrates	Total Lipids
7 (Reference)					
Pre-Spill	0.34	0.642 ± 0.037	11.68 + 0.77	3011 ± 1.20	158.08 ± 17.76
1st Post-Spill	114	1.272 ± 0.127	13.85:0.96	0.26 + 0.19	141.07 ± 15.29
2nd Post-Spill	47	1.608 ± 0.125	16.84 + 1.30	0.74:0.41	163.14 ± 22.02
9 (Dispersed Oil)					
Pre-Spill	0.38	0.742" + 0.044^{C}	10.32 + 0.64^{B}	1.12 + 0.58^{C}	148.39 ± 17.18
1st Post-Spill	168	0.722 ± 0.032^{ABC}	11.31 ± 1.02^{C}	2.11 ± 0.60	183.52 ± 10.21BC
2nd Post-Spill	124	1.517 ± 0.098	12.88 ± 1.40A	1.66 ± 0.52	166.01 ± 13.16
10 (Dispersed Oil)					
Pre-Spill	0.68	0.744 + 0.058^{C}	14.58 ± 1.02	0.13 + 0.07^{C}	170.31 ± 11.98
1st Post-Spill	322	0.428 ± 0.048A	17.39 ± 1.75	0.63 ± 0.25	133.40 ± 8.59
2nd 14 d Post-Spill	144	1.515 ± 0.140	14.50 ± 1.01	0.95 ± 0.29	215.50 ± 12.87
11 (Oil Alone)					
Pre-Spill	0.43	1.482 ± 0.155A	12.29 ± 0.99	1.67 ± 0.53	169.65 ± 12.47
1st Post-Spill	2.0	0.514 ± 0.075A	15.03 ± 0.60	0.27 ± 0.26	118.52 ± 12.10
2nd Post-Spill	93	1.459 ± 0.071	13.86 ± 1.02	1.34 ± 0.39	166.34 ± 18.42

A, Significantly cliff erent from Reference (Sta. 7), Student's T-test, or Kruskal-Wallis one-way ANOVA
B, Significant y dif ferent from Disp. Oil (Sta. 10), Student's T-test, or Kruskal-Wallis one-way ANOVA
C, Significantly dif ferent from Oil Alone (Sta. 11), Student's T-test, or Kruskal-Wallis one-way ANOVA

clams from Bay 9 in the second post-spill sample was the only value which was significantly different from the corresponding value in clams from the reference bay. Concentrations of total other carbohydrates, which consist of trehalose and other non-glycogen oligosaccharides and polysaccharides which yield glucose upon hydrolysis, were highly variable and no obvious trends among samples from different bays or sampling times were apparent.

Concentrations of total lipids in clams from Bays 7, 10 and 11 dropped between the pre-spill and first post-spill samples and then returned to pre-spill or higher values by the time of the second post-spill sampling. In clams from Bay 9, total lipid concentration increased between pre-spill and first post-spill and then dropped to the pre-spill range by the time of the second post-spill sampling.

The degree of contamination of Mya truncata with petroleum hydrocarbons varied greatly within each bay depending on the station at which clams were collected (see Section 2.). Data were available by station for petroleum hydrocarbon burden and tissue glucose and glycogen concentration in Mya truncata from the second post-spill collection (Table 4.2). Mean body burdens of petroleum hydrocarbons in clams from 30 stations in 4 bays ranged from 35 to 238 µg/g (ppm). However, there was no relationship between body burden of petroleum hydrocarbons and concentrations of glucose and glycogen in the tissues of clams. In Bays 9 and 10, clams were collected at both the 3-meter and 7-meter isobaths. There were no statistically significant differences between clams from the two depths in concentration in the tissues of petroleum, glucose, or glycogen.

Fourteen different free amino acids were identified and quantified in the adductor muscles of Mya truncata from the four bays. The mean concentration of total free amino acids ranged from 12.45 to 23.25 µM/mg wet weight in clams from different bays at different sampling times (Tables 4.3, 4.4, and 4.5). In clams from the two bays receiving dispersed oil (Bays 9 and 10), the mean concentration of tissue total free amino acids dropped between the pre-spill and first post-spill samples and then rose again in the second post-spill samples. The opposite trend was observed in clams from the bay receiving oil alone (Bay 11), while tissue total free amino acid concentrations in clams from the reference bay (Bay 7) remained relatively constant (range 15.37-17.65 µM/mg).

Table 4.2. concentrations of petroleum hydrocarbons, glucose and glycogen in tissues of truncate soft-shell clams* truncata collected at Stations 1-5 along the 7-meter isobath in four bays and stations 6-10 along the 3-meter isobath in two bays. Samples were taken during the second postspill sampling period. Oil concentrations are in **µg/g** (ppm) and glucose **and glycogen values are in mg/g wet weight, with a sample size of four.**

		Station									
		1	2	3	4	5	6	7	8	9	10
Bay 7	Oil	79	35	37	49	44					
	Glucose	1.25	1.46	1.90	2.17	1.17					
	Glycogen	16.82	18.65	20.08	17.03	11.58					
Bay 9	Oil	115	104	116	90	152	128	153	147	119	129
	Glucose	2.08	1.15	1.11	1.62	1.86	1.32	1.50	1.19	2.04	1.29
	Glycogen	15.83	12.11	11.84	14.21	7.46	20.69	9.21	11.78	9.76	15.96
Bay 10	Oil	173	238	167	125	111	104	193	131	139	107
	Glucose	2.31	1.22	1.37	1.58	1.27	0.96	0.98	1.40	2.58	1.47
	Glycogen	17.79	16.13	13.70	16.18	21.09	12.32	10.13	5.62	14.56	17.25
Bay 11	Oil	130	87	81	81	96					
	Glucose	1.46	1.15	1.63	1.28	1.78					
	Glycogen	13.84	12.30	15.57	12.12	15.47					

Table 4.3. Mean concentrations of free amino acids in adductor muscles of truncate soft-shell clams Mya truncata collected from the four BIOS experimental bays before the simulated oil spills. Values are in µM/mg dry wt. and are the mean and standard error from 9 to 13 replicate animals. The number of clams analyzed is given in parentheses.

Amino Acid	Bay 9	Bay 10	Bay 11	Bay 7
Taurine	1.114 ± 0.175(9)A	1.020 ± 0.085(13)	0.726 ± 0.069(10)	1.020 ± 0.173(10)
Aspartate	1.023 ± 0.152ABC	0.438 ± 0.070	0.480 ± 0.080	0.480 ± 0.080
Threonine	0.208 ± 0.025ABC	0.112:0.009	0.127 ± 0.019	0.123 ± 0.011
Serine	0.369 ± 0.040	0.277 ± 0.025A	0.216 ± 0.028A	0.402:0.061
Glutamate	-	0.392 ± 0.031A	0.425 ± 0.084	0.517 ± 0.053
Glycine	10.259 ± 1.250ABC	7.551 ± 0.558	7.098 ± 0.943	7.252:0.634
Alanine	2.675 ± 0.535	2.117 ± 0.223	1.728 ± 0.313A	2.811 ± 0.374
Valine				
Methionine				
Isoleucine				
Phen ylalanine	0.172 ± 0.056	0.145 ± 0.020	0.120 ± 0.021	0.125 ± 0.012
Histidine	0.917 ± 0.145ABC	0.457 ± 0.059	0.446 ± 0.061	0.415 ± 0.089
Lysine	0.194: 0.037AB	0.112 ± 0.012	0.140:0.022	0.125 ± 0.015
Arginine				
NH3	1.963 ± 0.874	1.825 ± 0.173	2.006 ± 0.236	2.102 ± 0.177
Mean Total Free Amino Aads	1S.894	14.446	13.510	15.372

A. Significantly different from Control (Bay 7) by Student's T-test or Mann-Whitney one-tailed U-test at $\alpha \leq 0.05$.

B. Significantly different from Bay 10 by Student% t-test or Mann-Whitney one-tailed u-test at $\alpha \leq 0.05$.

c. Significantly different from Bay 11 by Student's T-test or Mann-Whitney one-tailed U-test at $\alpha \leq 0.05$.

Table 4.4. Mean concentrations of free amino acids in adductor muscles of truncate soft-shell clams Mya truncata collected from the four BIOS experimental bays one to three days after the simulated oil spills. Values are in µM/mg dry wt. and are the mean and standard error from 7 to 20 replicate animals. The number of clams analyzed is given in parentheses.

Amino Acid	Bay 9	Bay 10	Bay 11	Bay 7
Taurine	0.999 + 0.1 10(20)	0.908 + 0.050(18)	2.007 + 0.001(10)	1.173 + 0.095(7
Aspartate	0.368 ± 0.051ABC	0.112 ± 0.025AC	0.716 ± 0.061A	0.595 ± 0.051
Threonine	0.119 ± 0.012	0.151 ± 0.012	0.124 ± 0.011	0.156 ± 0.030
Serine	0.253 ± 0.031	0.208 ± 0.015C	0.281 ± 0.021	0.267 ± 0.040
Glutamate	0.382:0.038	0.315:0.020	0.438:0.020	0.443:0.090
Glycine	7.311 ± 2.911	6.539 ± 0.305	7.640 ± 0.562	8.647 ± 0.848
Alanine	2.399:0.317	1.768:0.180	2.145 ± 0.288	2.643 ± 0.512
Valine				
Methionine				
Isoleucine				
Phenylalanine	0.125: 0.032B	0.044 ± 0.006AC	0.129 + 0.013	0.103 + 0.009
Histidine	0.342:0.071	0.311:0.019	0.47070.037	0.391:0.051
Lysine	0.104 ± 0.011AC	0.118 ± 0.006C	0.163:0.013	0.176 ± 0.025
Arginine				
NH_3	2.340 ± 0.206B	1.979 ± 0.071A	2.002 ± 0.196	2.549 ± 0.249
Mean Total Free Amino Acids	14.742	12.453	**16.115**	17.651

A. Significant y different from Control (Bay 7) by Student's T-test or Mann-Whitney one-tailed U-test at $\alpha \leq 0.05$.

B. Significantly different from **Bay** 10 by Student's T-test or Mann-Whitney one-tailed U-test at $\alpha \leq 0.05$.

c . Significantly different from Bay 11 by Student% T-test or Mann-Whitney one-tailed U-test at $\alpha \leq 0.05$.

Table 4.5. **Mean concentrations of free amino acids in adductor muscles of truncate soft-shell clams Mya truncata collected from the four BIOS experimental bays 14 days after the simulated oil spills. Values are in µM/mg dry wt. and are the mean and standard error from 10 to 20 replicate animals. The number of clams analyzed is given in parentheses.**

Amino Acid	Bay 9	Bay 10	Bay 11	Bay 7
Taurine	1.021 + 0.011(20Y3	3.924 + 0.550(16)AC	0.771 ± 0.053(10)	0.944 + 0.049(10)
Aspartate	0.091: 0.012C	O. 106 ± 0.029C	0.171 ± 0.013A	0.101 ± 0.010
Threonine	0.249 ± 0.035B	0.060 ± 0.018AC	0.157 ± 0.018	0.266 ± 0.109
Serine	0.284 ± 0.034B	0.115 ± 0.026	0.21270.109	0.213:0.011
Glutamate	0.454 ± 0.052B	0.293 ± 0.028AC	0.403 ± 0.032	0.425 ± 0.028
Glycine	9.132: 1.070B	12.912: 1.91oAC	6.946 ± 0.450	8.769 ± 0.350
Alanine	2.130 ± 0.254ABC	0.886 ± O. 120AC	1.538 ± 0.177A	2.866 ± 0.263
Valine	0.121 ± 0.010	0.068: 0.024AC	0.125:0.018	0.097:0.008
Methionine	0.048 ± 0.005B	0.761:0.01 4AC	0.04570.010	0.03370.004
Isoleucine	0.065 ± 0.013	0.052 ± 0.019	0.086:0.015	0.010 ± 0.006
Phen ylalanine	0.072 ± 0.020	0.053 ± 0.011	0.064:0.017	0.07070.019
Histidine	0.365: 0.024BC	0.163 ± 0.016AC	0.282 ± 0.015A	0.40170.015
Lysine	0.182 ± 0.022B	0.087 ± 0.026AC	o.157 ± 0.022	0.140 ± 0.010
Arginine	0.396: 0.049B	2.616: 0.540AC	0.310:0.026	0.382 ± 0.023
NH3	1.707 ± 0.169B	1•154 ± 0.099C	1.738 ± 0.141A	1.374:0.091
Mean Total Free Amino Acids	**16.317**	23.250	13.005	**16.141**

A. Significant y cliff erent from Control (Bay 7) by Student's T-test or Mann-Whitney one-tailed U-test at a <0.05.

B. Significantly different from Bay 10 by Student's T-test or Mann-Whitney one-tailed U-test at $\alpha \leq 0.05$.

c. Significantly different from Bay 11 by Student's T-test or Mann-Whitney one-tailed U-test at $\alpha \leq 0.05$.

In clams collected immediately before the BIOS oil spills, concentrations of several tissue free amino acids were significantly different in clams from the four bays (Table 4.3). The mean concentration of 6 free amino acids was significantly different in clams from the reference bay (Bay 7) and Bay 9, while concentrations of only two amino acids in clams from Bays 10 and 11 were significantly cliff erent from those of clams from the reference bay. Immediately after the spills, concentrations of 1-3 amino acids were significantly different in clams from the reference bay and from the three bays receiving dispersed or undispersed crude oil (Table 4.4). Concentrations of total and most individual tissue free amino acids were lower in clams from the most heavily contaminated bay (Bay 10) than in clams from the other two bays receiving oil and the reference bay. In clams collected during the second post-spill sampling approximately 2 weeks after the spills, there were many statistically significant differences in concentrations of individual tissue free amino acids among clams from the four bays (Table 4.5). Values for clams from Bay 10 [the most heavily contaminated bay) varied most from the corresponding values for clams from the other three bays.

Two parameters which have been recommended as indices of sublethal stress in marine invertebrates are the molar ratio of taurine to glycine and the sum of the concentrations of threonine plus serine. Stressed animals should have a higher taurine/glycine ratio and lower threonine plus serine concentration than unstressed animals. The only oil-exposed group of clams with taurine/glycine ratio and threonine plus serine concentration significantly different from that of clams from the reference bay was that from the most heavily contaminated bay (Bay 10) collected during the second post-spill sampling (Table 4.6). Taurine/glycine ratio was elevated and threonine plus serine concentration was depressed relative to reference animals.

Spearman rank correlation tests were performed on all biochemical parameters measured for clams from the three sampling times and four experimental bays. Parameters which showed a high ($\alpha < 0.05$) degree of interassociation, positive or negative, are tabulated according to sampling time and inter-bay association in Table 4.7. Clams from the second post-spill sampling had the largest number of associated pairs of biochemical parameters (Table 4.8). One-hundred and five associated pairs were shared by all four bays, indicating that in these samples, clams from the four bays were very uniform in relative (though not necessarily absolute) values for the biochemical

Table 4.6. Molar ratio of taurine to glycine and the sum of the concentrations of threonine plus serine in the free amino acid pool of adductor muscles of truncate soft-shell clams **Mya truncata** from the **four BIOS experimental bays. Concentrations of threonine plus serine are in µM/mg dry weight and are the mean and standard error of 7 to 20 replicate animals per treatment. The number of clams analyzed is given in parentheses.**

Parameter	Pre-Spill	1st Post-Spill	2nd Post-Spill	Pre-Spill	1st Post-spill	2nd Post-Spill
	Bay 9			Bay 10		
Taurine/Glycine	0.108 ± 0.007AB (9)	0.134 ± 0.006C (19)	0.109 + 0.006B (20)	0.137 + 0.007 (13)	0.141 + 0.007C (is)	0.297 ± 0.036AC (16)
Threonine + Serine	0.577 + 0.065BC (9)	0.365 + 0.040 (m)	0.533 + 0.067B (20)	0.389 + 0.033A (13)	0.359 + 0.024 (18)	0.191 ± 0.047AC (13)
	Bay 11			Bay 7		
Taurine/Glycine	0.109 + 0.007AB (TO)	0.263 + 0.109 (10)	0.112 + 0.006 (10)	0.141 + 0.012 (10)	0.141-1-0.012 (7)	0.109 + 0.006 (ii))
Threonine + Serine	0.343 + 0.045A (10)	0.424 + 0.035 (in)	0.384 + 0.038 (10)	0.525 + 0.070 (10)	0.367 + 0.056 (7)	0.479 + 0.104 (10)

A. Significantly different from Control (Bay 7) by Student's T-test or Mann-Whitney one-tailed U-test at $\alpha \leq 0.05$.

B. Significantly different from Bay 10 by Student's T-test or Mann-Whitney one-tailed U-test at $\alpha \leq 0.05$.

c. Significantly different from Bay 11 by Student's T-test or Mann-Whitney one-tailed U-test at $\alpha \leq 0.05$.

Table 4.7. A summary of associated pairs of biochemical parameters in *Mya truncata* determined by the Spearman Rank Correlation Test. Data are tabulated by pairs shared among bays and by sampling times.

	Taurine	Aspartate	Threonine	Serine	Glutamate	Glycine	Alanine	Valine	Methionine	Isoleucine	Phenylalonine	Histidine
Taurine												
Aspartate	3A											
Threonine	**2B,3A**	3A										
Serine	**1E,3A**	3A	**1B,3A**									
Glutamate	**3A**	3A	3A	3A								
Glycine	**1E,3A**	**1A,3A**	**1A,3A**	**1A,3A**								
Alanine	**1E,3A**	3A	**1A,3A**	**1A,2B,3A**	**1C,3A**	**1E,3A**						
Wine	3A	3A	3A	3A	3A	3A	3A					
Methionine	3A	3A	3A	3A	3A	3A	3A	3A				
Isoleucine	3A	3A	3A	3A	3A	3A	3A	3A				
Phenylalanine	3A	**3A**	3A	**1C,3A**	**3A**	3A	3A	3A	3A	**3A**		
Histidine	3A	**3A**	3A	3A	**3A**	3A	3A	3A	3A	**3A**	3A	
Lysine	3A	3A	**1E,3A**	**2B,3A**	3A	3A	**1 E,3A**	3A	3A	3A	3A	**1 B,3A**
Arginine	3A	3A		**3A**	**3A**	3A	3A	3A	3A	3A	3A	3A
NH_3	**3A**	3A		**1 A,3A**	**1C,3A**	**1E,3A**	3A	3A	3A	3A	3A	3A
Total AA			**1C**	1A			**1C**				**1C**	**1B**
Taurine/Glycine												
Threonine + Serine	**1E**		**1A,2B**	1A			**1E**					**1B**
Glycogen												
Glucose												
other **Carbons**												
Lipids												

	Lysine	Arginine	NH3	Total AA	Taurine/Glycine	Threonine + Serine	Glycogen	Glucose	Other Carbons	Lipids
Taurine										
Aspartate										
Threonine										
Serine										
Glutamate										
Glycine										
Alanine										
Valine										
Methionine										
Isoleucine										
Phenylalanine										
Histidine										
Lysine										
Arginine	3A									
NH3	3A	3A								
Total AA	**1E**									
Taurine/Glycine										
Threonine + Serine	**1B**			**1C**						
Glycogen										
Glucose										
other carbons										
Lipids							30			

Sampling Period* 1, pre-spill; 2, first post-spill; 3, second post-spill.
Bay Associations A, Bays, 7,9,10,11; B, Bays 7,9,10; C, Bays 7,9,11; D, Bays 7,10,11; E, Bays 9,10,1 I

Table 4.S. The number of associated pairs of biochemical parameters in the truncate clam Mya truncata collected from the BIOS experimental bays at three sampling times. Bay combinations denote paired associations which are shared.

Bay Combinations	Pm-spill	First Post-Spill	Second Post-Spill
7,9,10,11	9		105
7,9,10	5	5	
7,9,11	7		
7,10,11			1
9,10,11	10		
7,9	9		
7,10			
7,11			
9,10	16		
9,11	11		
10,11	1		
7	1	5	
9	26	36	
10	1	4	
11	1		
Total Associated Pairs	**97**	**68**	**106**

parameters measured. Clams from the first post-spill sampling had the lowest number of associated pairs and the greatest inter-bay diversity. Clams from the pre-spill sample were intermediate. At all three sampling times, there was little association among values for carbohydrate, lipid and free amino acid parameters. In clams from the first post-spill sampling, there were no associated pairs shared by Bay 11 (receiving oil alone) and the other three bays.

4.3 Discussion

There was a high degree of variability in the values for different biochemical parameters in replicate clams from the same sample, among samples from different bays, and in samples collected at different times. This variability makes it difficult to identify biochemical responses of clams to the oil spills. There are several possible explanations for the observed variability.

Bivalve molluscs, like many other marine invertebrates, typically show a wider range of normal (unstressed) values for many biochemical parameters than do fish and other "higher" animals (Newell, 1976; Gabbott, 1976; Carr and Neff, 1981, 1982). In species such as the mussel Mytilus edulis for which an extensive body of basic biochemical and physiological information is available (Bayne, 1976), some of this variability can be accounted for or controlled. There are practically no data available on the normal biochemistry, physiology, and seasonal cycles of Mya truncata.

Perhaps more important, and a major problem in a remote field experiment of this sort, are the methods used to sample and handle animals in the field. Mya from the second post-spill sampling were much more uniform in all biochemical parameters measured than were clams from the first two collections. It is quite possible that this was due in part to cliff erences in handling of the animals by the field collecting teams. A substantial time delay between collecting the clams and freezing them can result in large and unpredictable changes in several of the biochemical parameters studied, particularly concentrations of tissue glucose and free amino acids. Ideally, samples should be frozen in liquid nitrogen or dry ice immediately upon collection. This was not feasible in the BIOS study. Although samples apparently were frozen within a few hours of collection in most cases, notes in the collecting log book indicate that some samples were held

overnight or even for several days in a refrigerator before freezing. The most variable set of samples was that from the first post-spill collection. Examination of the field log book indicated that these samples were collected over a 10-day period (from Bay 9 on 8/28, 29 and 31/81; from Bay 10 on 8/29-30/81; from Bay 11 on 8/21/81; and from Bay 7 on 8/31/81). The simulated spill of oil alone in Bay 11 was on 8/19/81 and the simulated spill of dispersed oil in Bay 9 was on 8127/81. Thus, clams from the bay receiving oil alone (Bay 11) were sampled two days after the spill, while those from bays receiving dispersed oil were sampled up to four days after the spill. Thus, it is difficult to compare acute responses of clams to the different treatments. Collection of the pre-spill and second post-spill samples also took place over several days, but the interpretive problem in these cases is less severe. It also should be pointed out that samples for hydrocarbon analysis were not always taken at the same time as samples for biochemical analysis at a given bay and station.

Despite these problems, some conclusions can be drawn f rom the results of these biochemical studies on Mya truncata. Based on results of the biochemical analyses, truncate soft-shell clams were not severely stressed by either dispersed or undispersed oil at the contaminant levels attained in the BIOS experiment. Although all treatment groups were exposed to and subsequent y accumulated some petroleum, and therefore there was no true control or reference group of animals, clams from Bay 7 were the least heavily contaminated. Therefore, they can be used, in lieu of a true reference. Clams from Bay 11 (undispersed crude oil) differed the most from clams from Bay 7, particularly in the second post-spill sample. Clams from Bay 10 [dispersed crude oil) became more heavily contaminated with petroleum hydrocarbons than clams f rom the other dispersed oil bay (Bay 9) and showed greater differences than the latter in several biochemical parameters, as compared to clams from Bay 7. These differences were most marked in the first post-spill survey. Thus, we can conclude that chemically dispersed oil may cause more severe acute effects than undispersed oil in benthic infaunal molluscs, but longer-term impacts of undispersed crude oil may be more severe than those of chemically dispersed oil. This is undoubtedly related to the observations documented in the Section 2 of this report that petroleum. contamination of filter-feeding molluscs was greatest in the bays receiving dispersed oil and reached a peak in the first post-spill samples, decreasing in the second post-spill samples. On the other hand, contamination of clams in the bay receiving oil

alone was more gradual and reached a peak in the second post-spill sample. Undispersed crude oil may be more persistent than chemically dispersed oil in bottom sediments and so lead to more serious long-term effects. We have obtained similar results in recent mesocosm experiments with chemically dispersed oil (Neff, 1982). Benthic animals in tanks receiving chemically dispersed crude oil experienced higher short-term mortality and sublethal effects than animals receiving oil alone. However, after a month, sublethal physiological and biochemical responses were more marked in animals from the undispersed oil treatment groups than the dispersed oil treatment groups.

In this investigation, several biochemical parameters were evaluated as indices of pollutant stress in truncate soft-shell clams exposed to dispersed and non-dispersed crude oil in the BIOS experiment. The parameters used were chosen based on their proven utility for this purpose, and because they could be measured in frozen samples, an important consideration considering the remoteness of the sampling site and lack of facilities to make measurements on-site on fresh tissues. Values for some of the biochemical parameters were significantly different in the four populations of Mya samples. Tissue free amino acid concentrations and ratios showed the most changes. Tissue free amino acids also were the most useful index of pollutant stress in oysters Crassostrea gigas from bays contaminated with crude oil from the Amoco Cadiz crude oil spill (Nef f and Haensly, 1982). It is possible that other parameters would have exhibited more significant differences than they did if there had been better control of the sampling and sample handling in the field.

4.3.1. **Weight-Length Relationships of Bivalves.** Cross and Thompson (1982) and Cross et al. (1983) have performed analyses of dry weight-shell length relationships of four species of bivalve molluscs from the four bays. Samples of up to 50 individuals each of Mya truncata, Macoma calcarea, Astarte borealis and Serripes groenlandicus were collected along the middle transect at the 7-meter depth in each bay on five sampling occasions (pre-spill, September, 1980 and August, 1981; post-spill, September) 1981P August 1982, September, 1982).

The investigators found evidence that weight-length relationships in Serripes groenlandicus and Macoma calcarea were affected by the experimental oil spills. The other species were unaffected. Larger specimens of S. groenlandicus from Bay 7 showed a progressive decrease in dry weight of soft tissues adjusted to a standard shell length from

the second pre-spill sample (immediate pre-spill sample in this investigation) to the third - post-spill sample (September, 1982). No progressive changes in adjusted dry weights or weight-length regressions were observed in S. groenlandicus from the other bays. A decrease in weight per unit shell length or adjusted to a standard shell length indicates a decrease in the condition or nutritional status of the mollusc. Although Bay 7 was considered a reference bay, it did receive 50-100 ppb of dispersed oil in the first few days after the discharge (Green et al., 1982). S. groenlandicus from the 7-meter depth in Bay 7 accumulated hydrocarbons to higher levels immediately after the spill than did the same species from the 7-meter depth in the other bays (See Section 2 of this report). S. groenlandicus from Bays 9 and 10, which received much higher levels of dispersed oil, probably were narcotized and/or stopped filtering, and therefore became less contaminated than animals from Bay 7. S. groenlandicus differed from the other filter-feeding mollusc studied, Mya truncata, in that it preferentially retained in its tissues a high molecular weight saturated hydrocarbon assemblage as well as the toxic highly alkylated naphthalenes, phenanthrenes, and dibenzothiophenes. These observations may partially explain the apparent impact of oil on S. groenlandicus from Bay 7.

Whereas S. groenlandicus is a filter-feeder and accumulates petroleum hydrocarbons primarily from the water, Macoma calcarea is a deposit-feeder and accumulates petroleum hydrocarbons primarily from contaminated sediments. Thus, as reported in the bioaccumulation section of this report, M. calcarea from Bay 7 did not accumulate significant body burdens of hydrocarbons because very little of the water-borne hydrocarbons entering the bay were deposited in the sediments. In the other three bays, substantial amounts of oil were deposited in bottom sediments and M. calcarea became the most heavily contaminated. Hydrocarbon body burdens in the deposit-feeders increased between the first and second post-spill samplings. Cross and Thompson (1982) and Cross et al. (1983) reported that M. calcarea from Bay 7 underwent a seasonal cycle of increasing length-adjusted tissue dry weight between August and September in both 1981 and 1982. This probably represented a natural cycle of fattening and gonadal maturation in the animals. However, M. calcarea from the other bays did not show evidence of this cycle, and in clams from Bay 9, there actually was a decrease in length-adjusted tissue dry weight between August, 1981 (pre-spill) and September, 1981 (second post-spill sampling). These results suggest that petroleum contamination of sediments

interfered with feeding, gonadal development! and bioenergetics of M. calcarea. Similar responses have been reported in bivalve molluscs impacted by the Chedabucto Bay, Nova Scotia oil spill (Gilfillan and Vandermeulin, 1978) and the Amoco Cadiz oil spill in Brittany, France (Neff and Haensly, 1982). interestingly, M. calcarea from Bay 9 did not have an elevated incidence of histopathological lesions compared to clams from other bays. M. calcarea from Bay 11 (receiving undispersed crude oil) did have an increased incidence of parasitism and hemolytic infiltration, and one specimen had a blood neoplasm. One year after the spill, these clams had a high incidence of vacuolization of the digestive tubule epitheliums, a pathological condition also reported in bivalve molluscs transplanted to a site heavily contaminated by the Amoco Cadiz oil spill (Wolfe et al., 1981).

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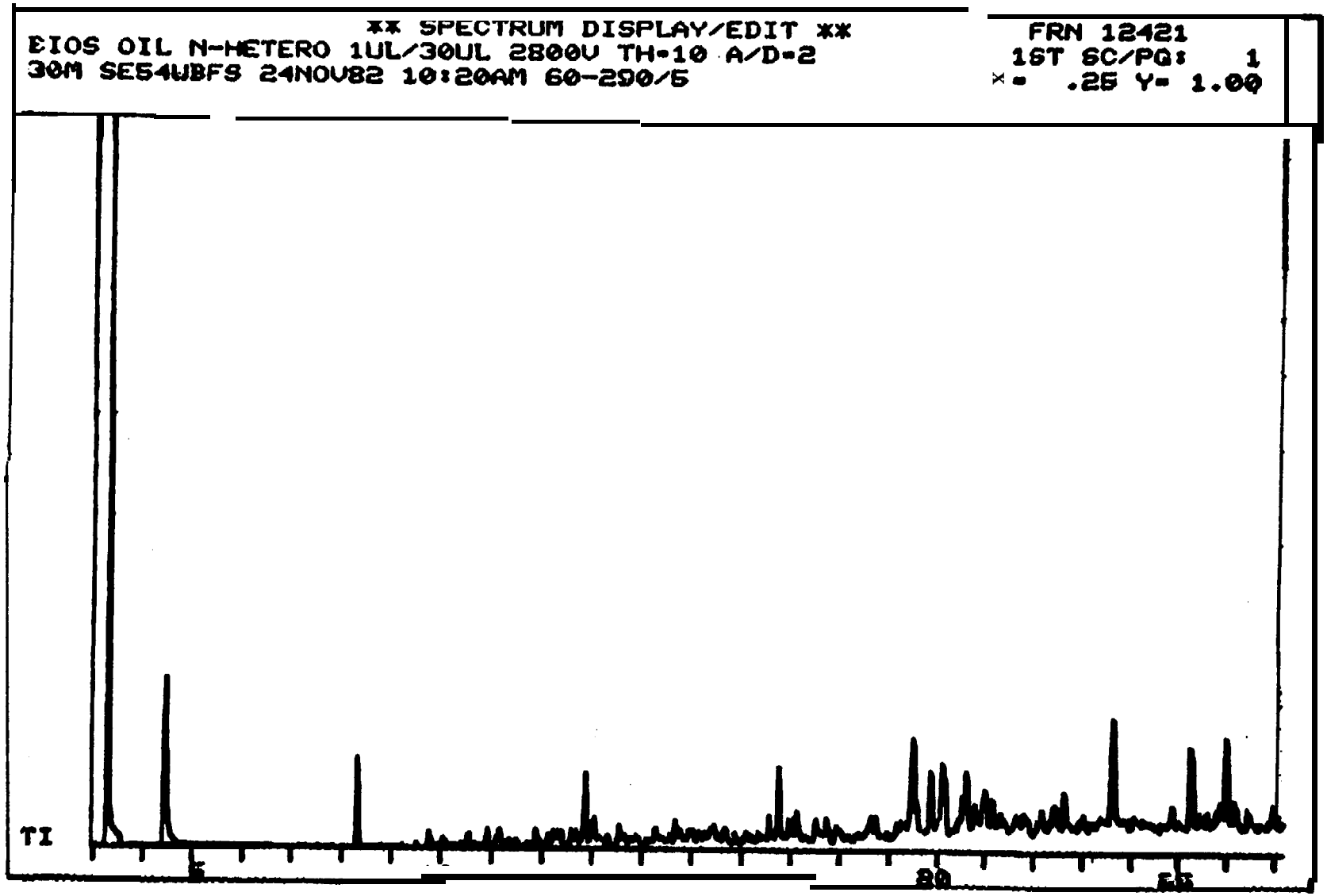
APPENDIX 1:

PANH COMPOUNDS IN OIL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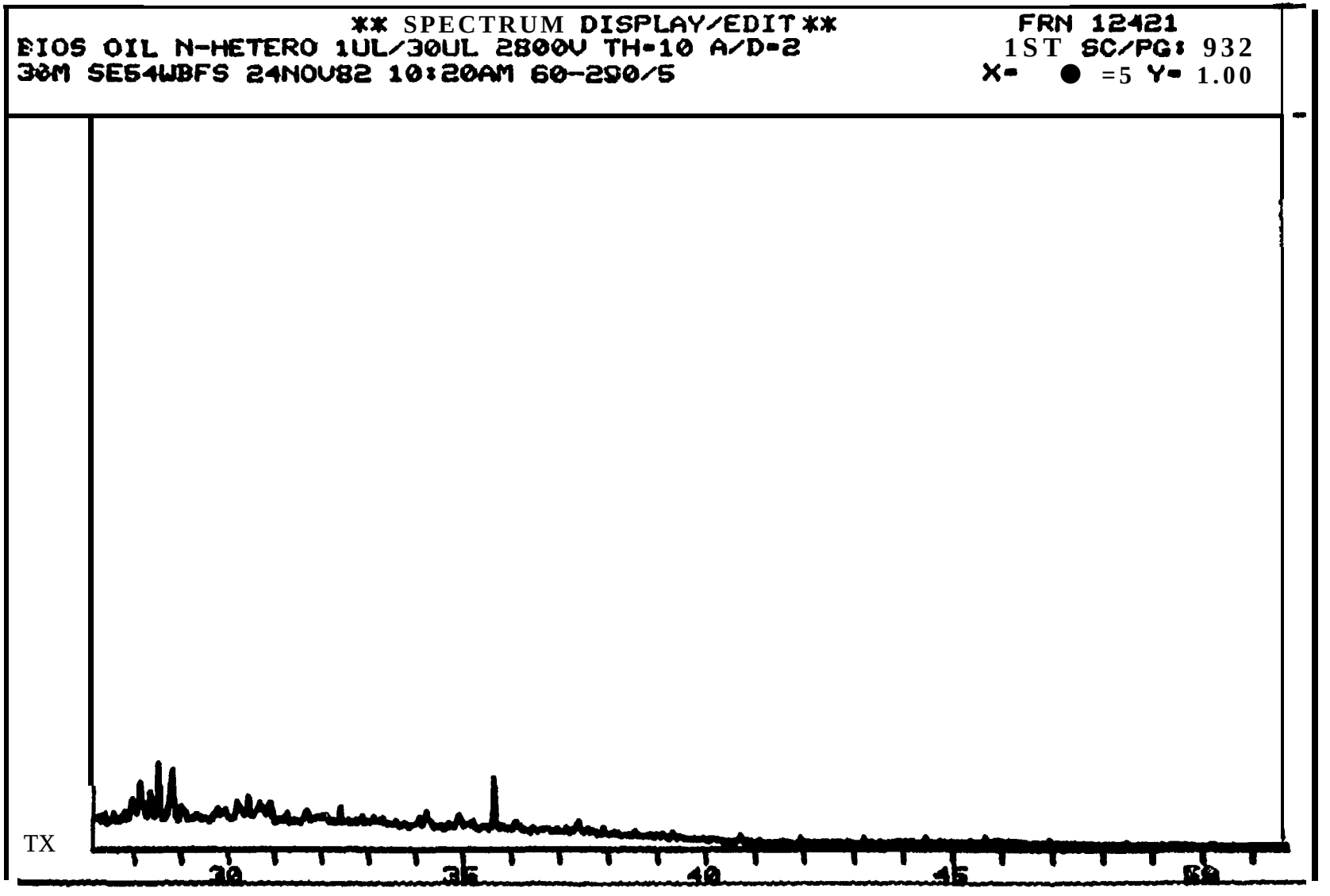
TI
80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TX
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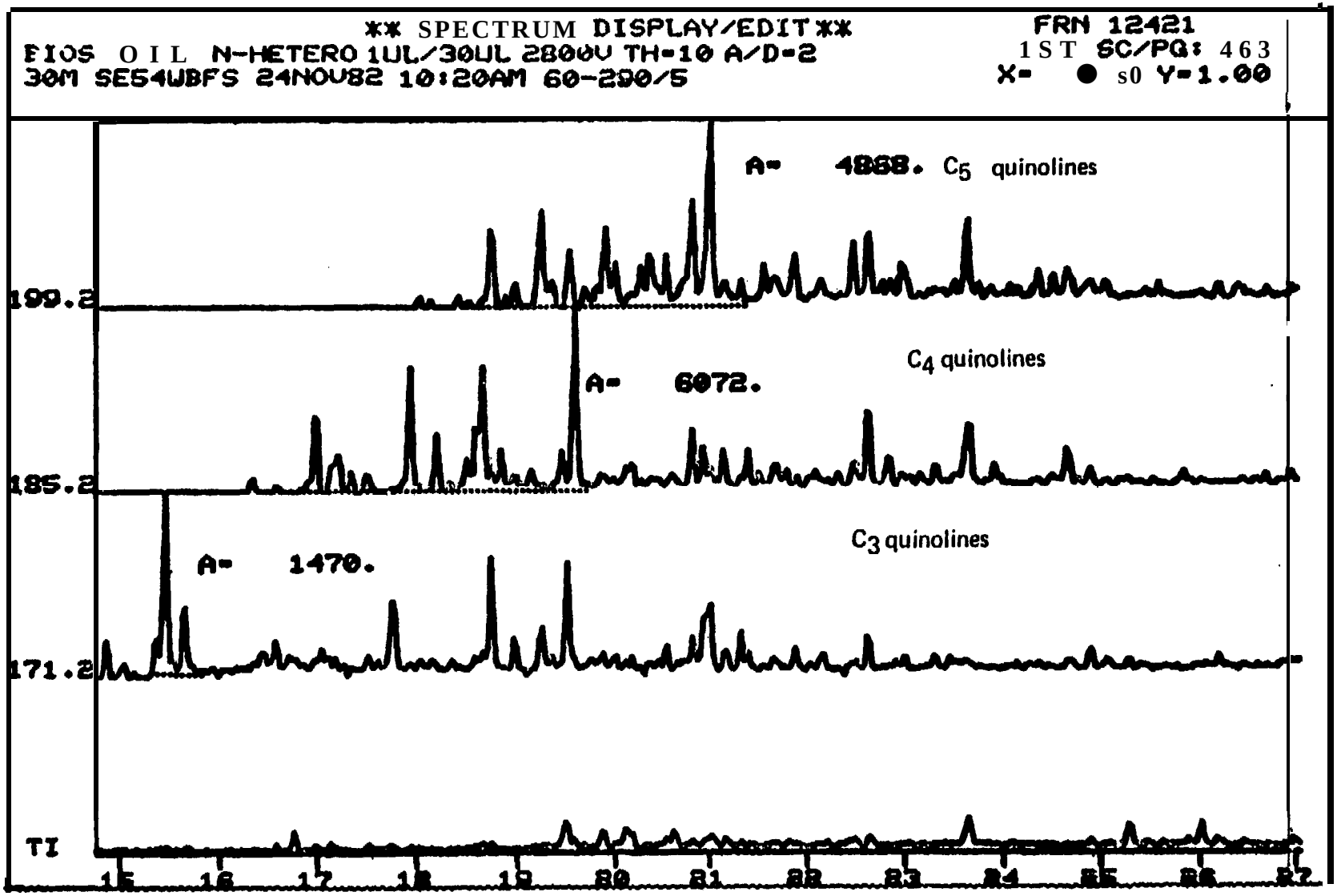
35
40
45
50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X= ● s0 Y=1.00
A= 4868. C5 quinolines
199.2
C4 quinolines
A= 6072.
185.2
C3 quinolines
A= 1470.
171.2
TI
15
16
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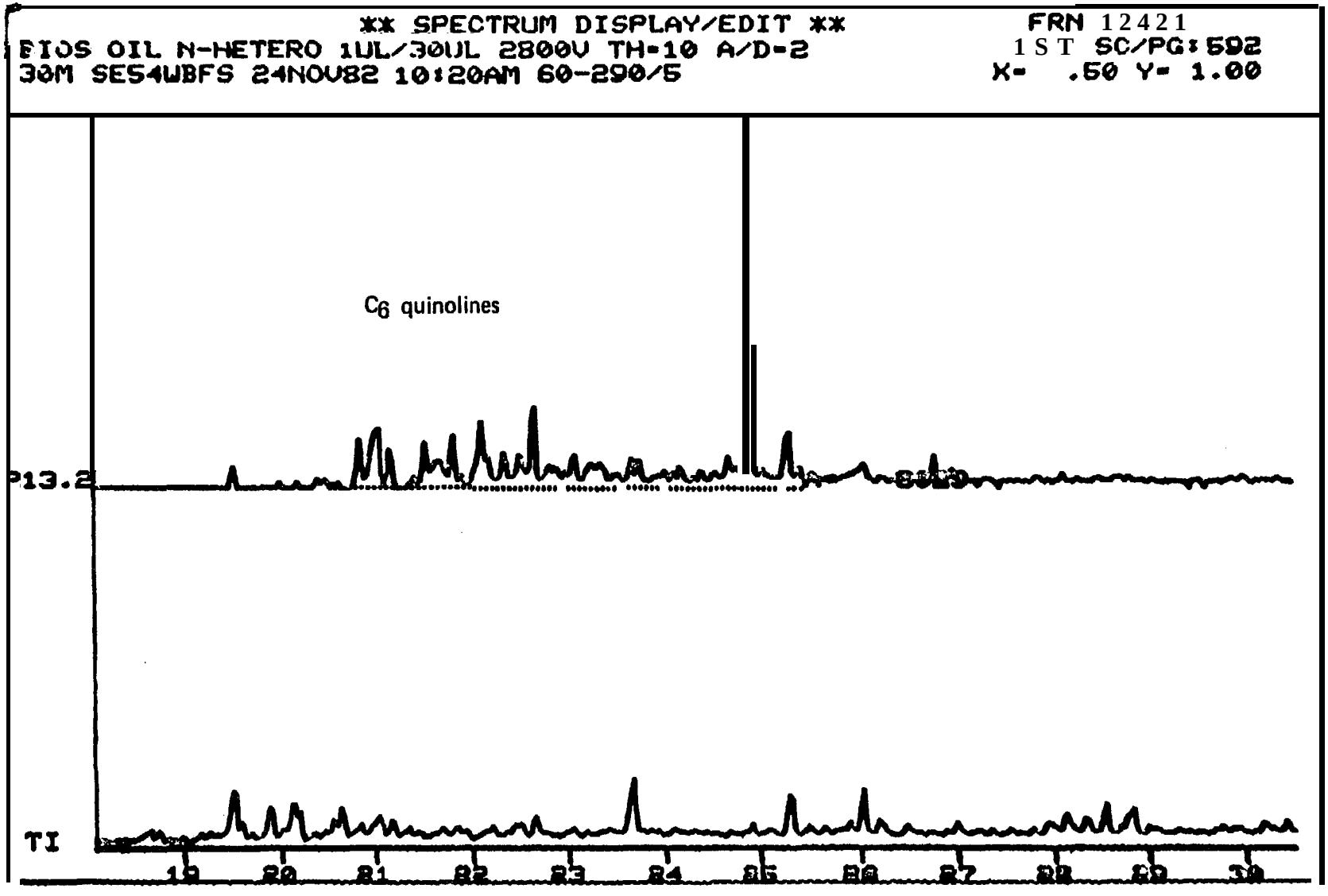
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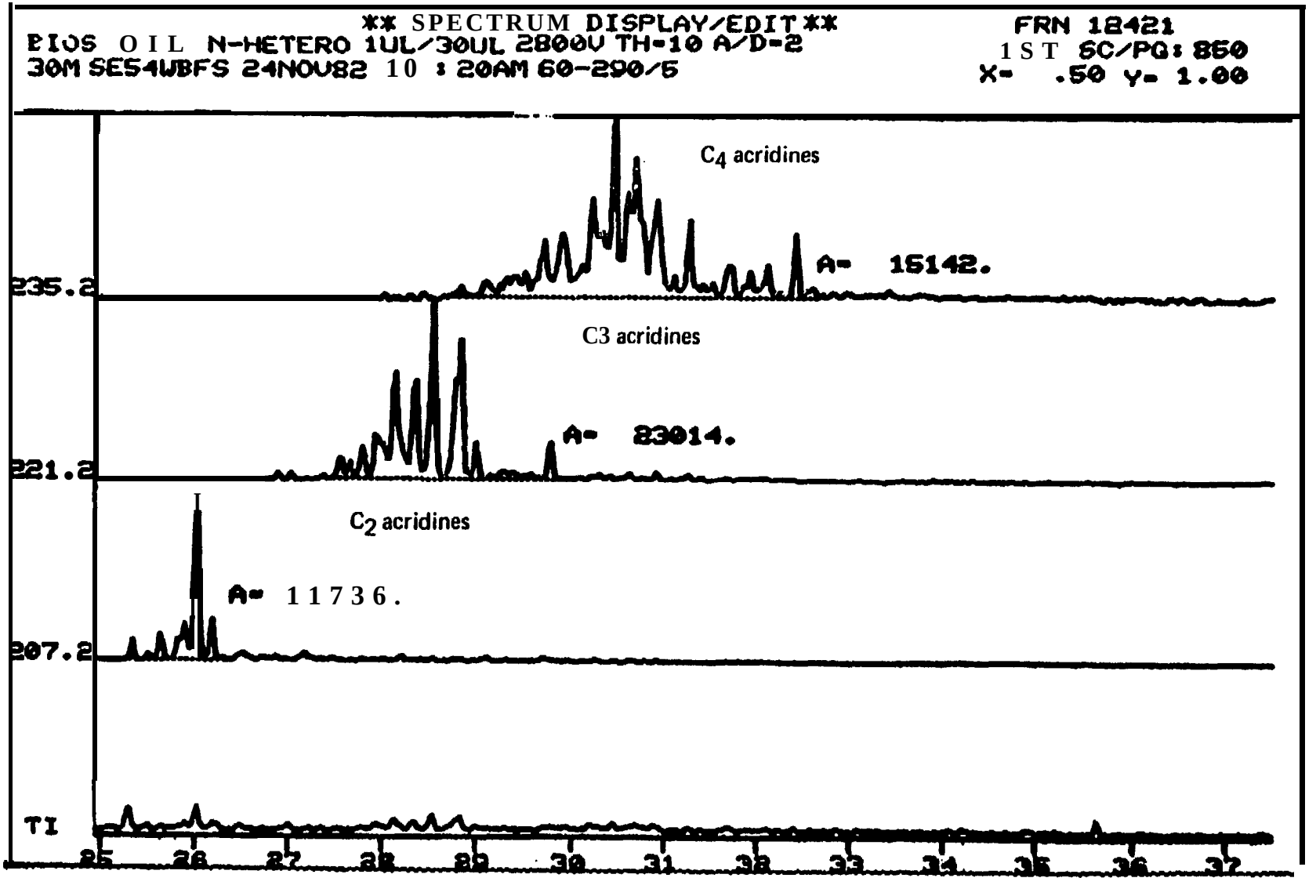
X= .50 Y= 1.00
C6 quinolines
TI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X- .50 Y- 1.00
C4 acridines
A- 15142.
235.2
C3 acridines
A- 23014.
221.2
C2 acridines
A- 11736.
207.2
TI
25
26
27
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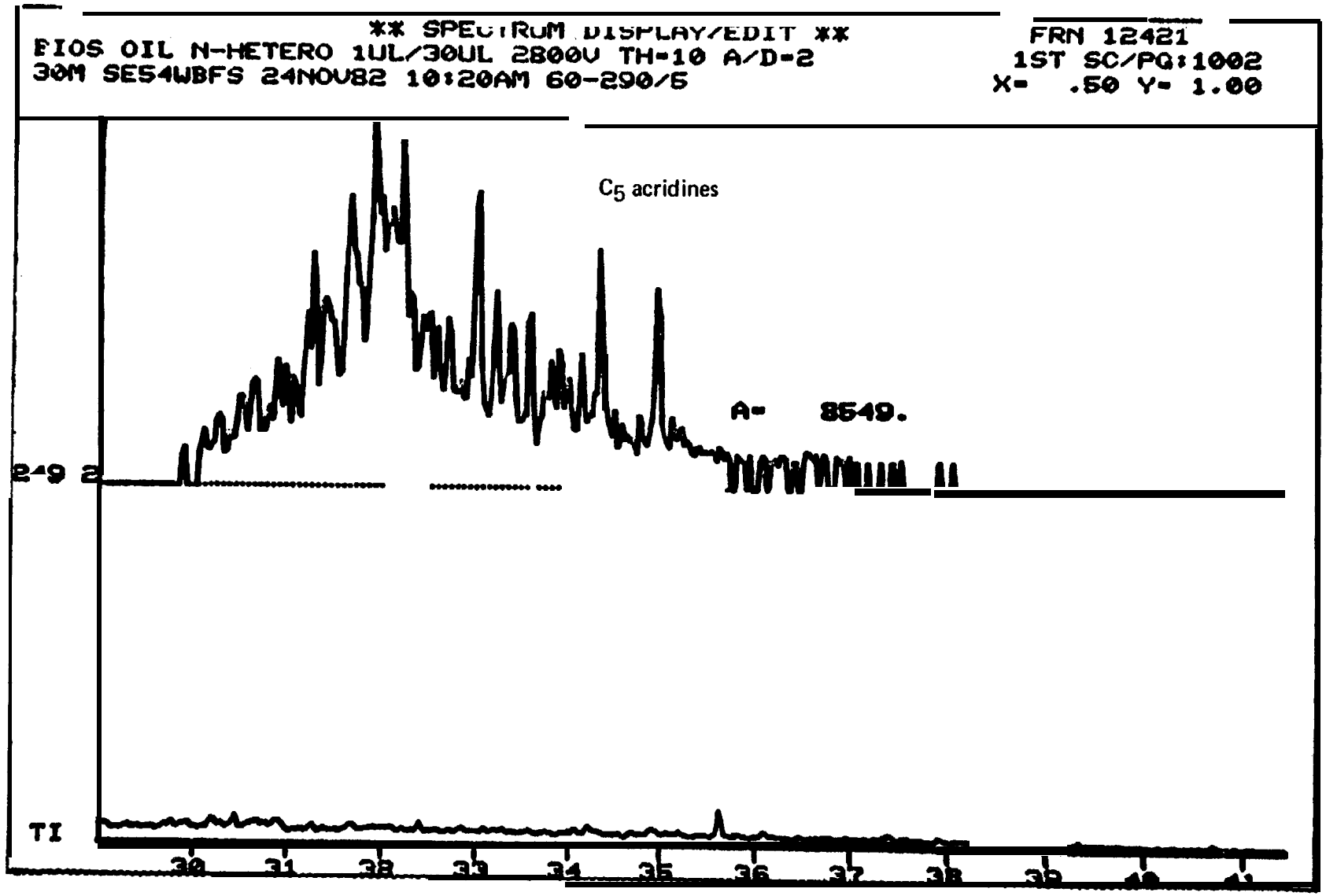
33
34
35
36
37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X= .50 Y= 1.00
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A= 8549.
249 2
TI
30
31
32
33
34
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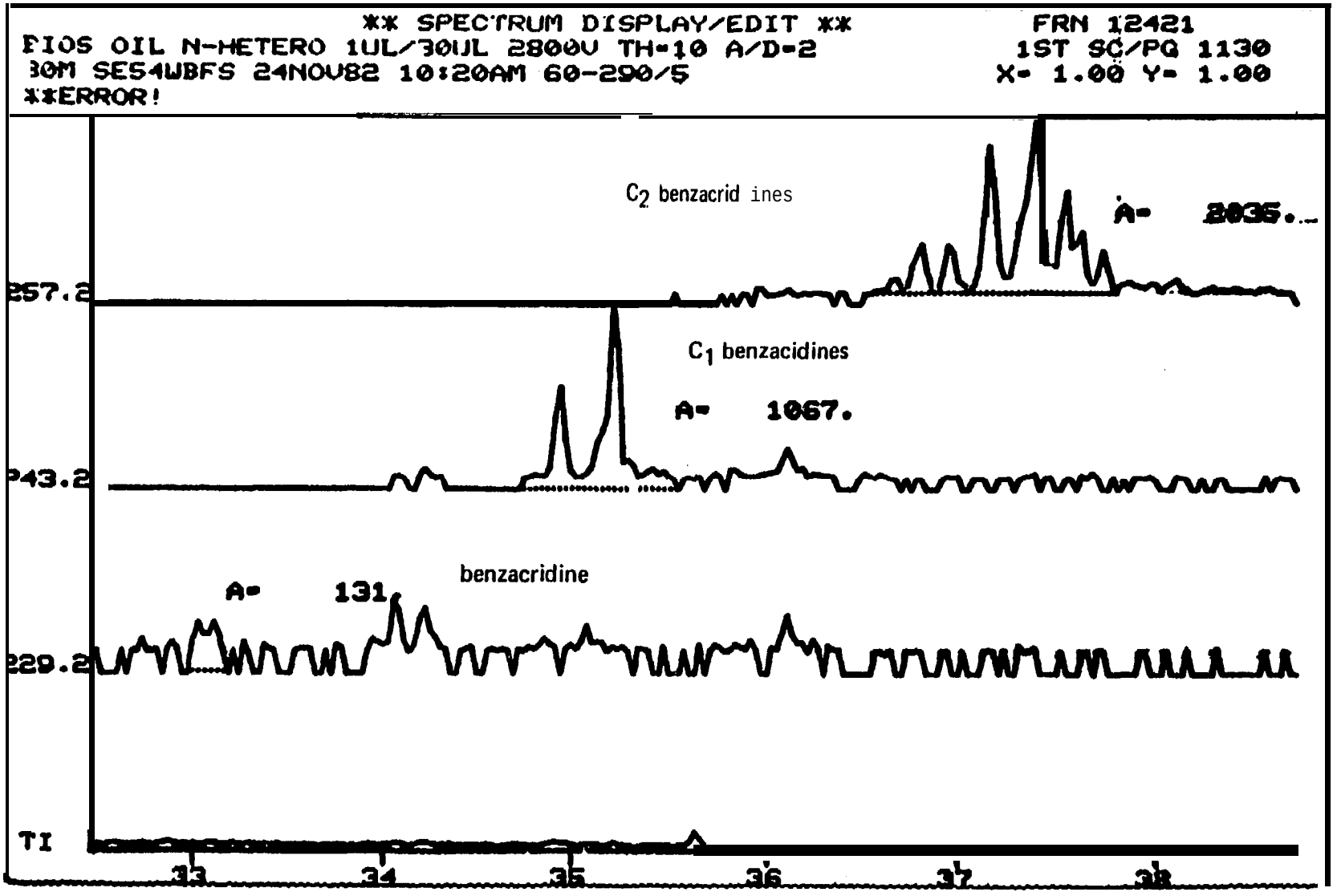
37
38
39
40
41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**ERROR!
C2 benzacrid ines
A= 2035.
257.2
C1 benzacidines
A= 1067.
243.2
benzacridine
A= 131
229.2
TI
33
34
35
36
37
38

FILE NUMBER 12421

ENTRY	TIME	MASS	AREA	%
1	1s".s	171.2	1470.	1.86
2	19.6	185.2	60?2 .	7.?0
3	21.0	199.2	4860.	6.17
4	24.9	213.2	8029.	10.18
5	26.0	207.2	1173s.	14.88
6	28*S	221.2	23014.	29.18
7	30.5	235.2	15142.	19.20
8	31.9	249-2	8549.	10.84

CAL % ON ENTRY?

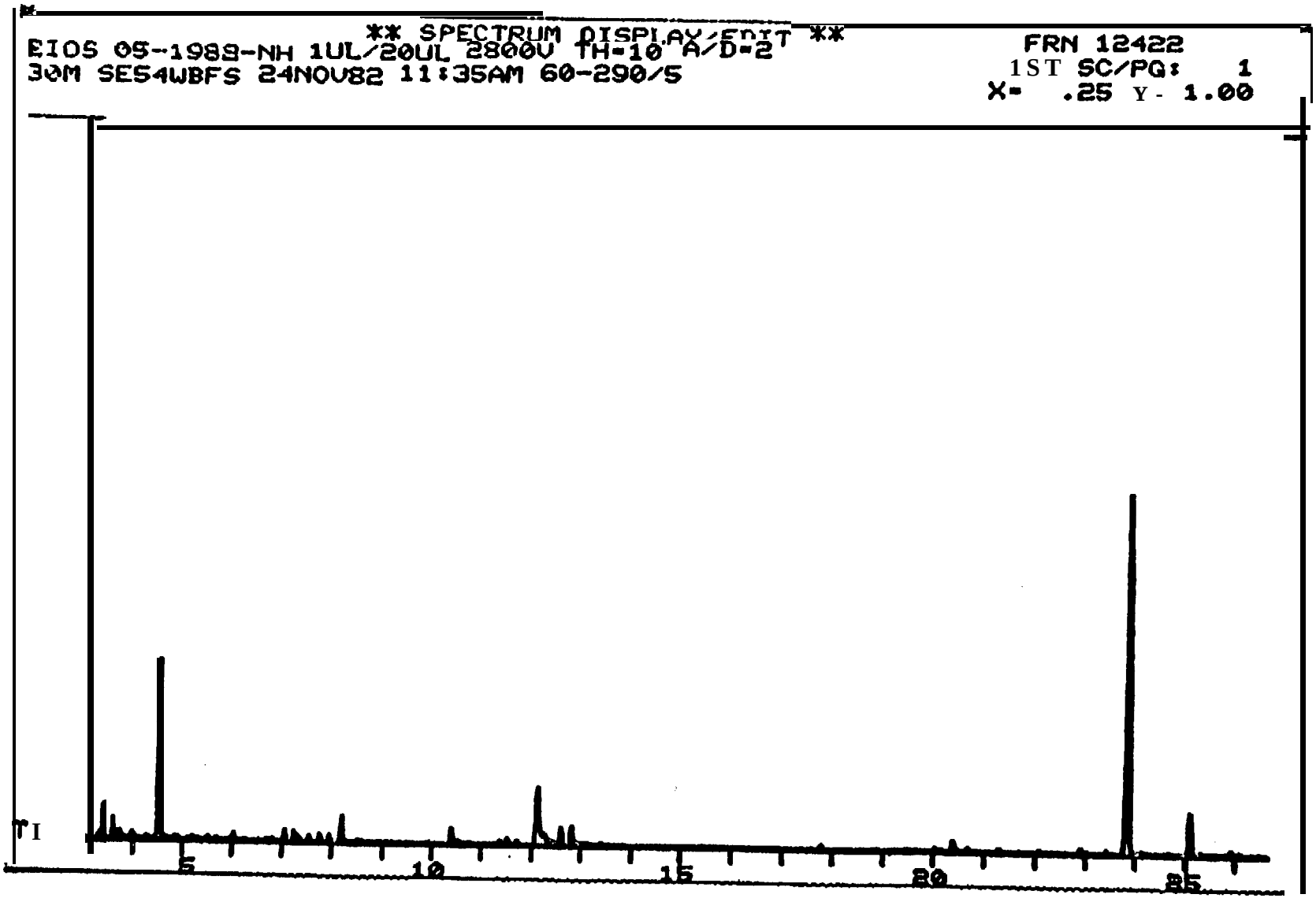
FILE NUMBER12431

ENTRY	TIME	MASS	AREA	%
1	33.0	229.2	131 .	4.04
2	35.2	243 .2	1067.	33.01
3	37*4	257.2	2035.	62.95

CAL % ON ENTRY?

APPENDIX II:

ASTARTE BOREALIS: BAY 9: 1 DAY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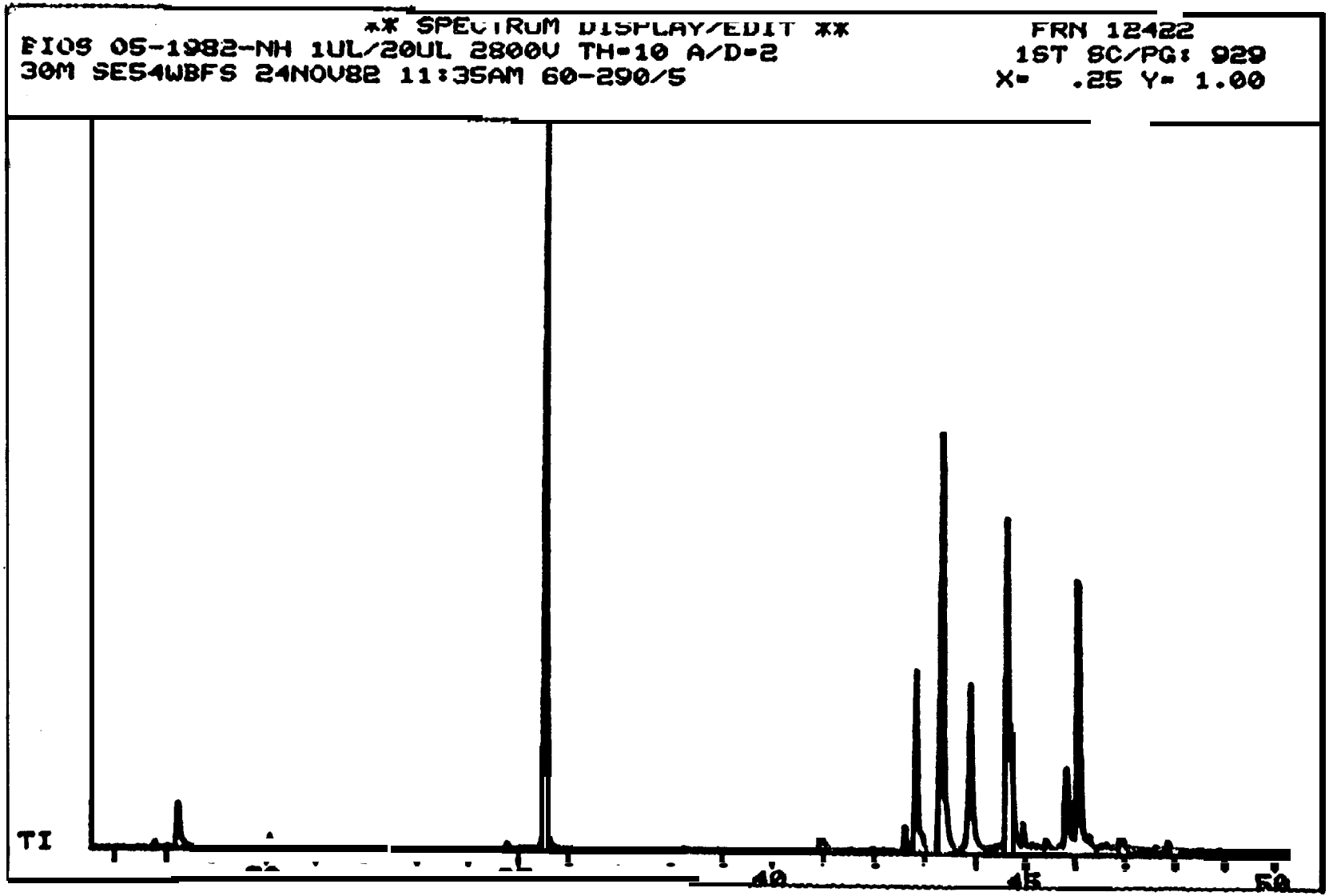
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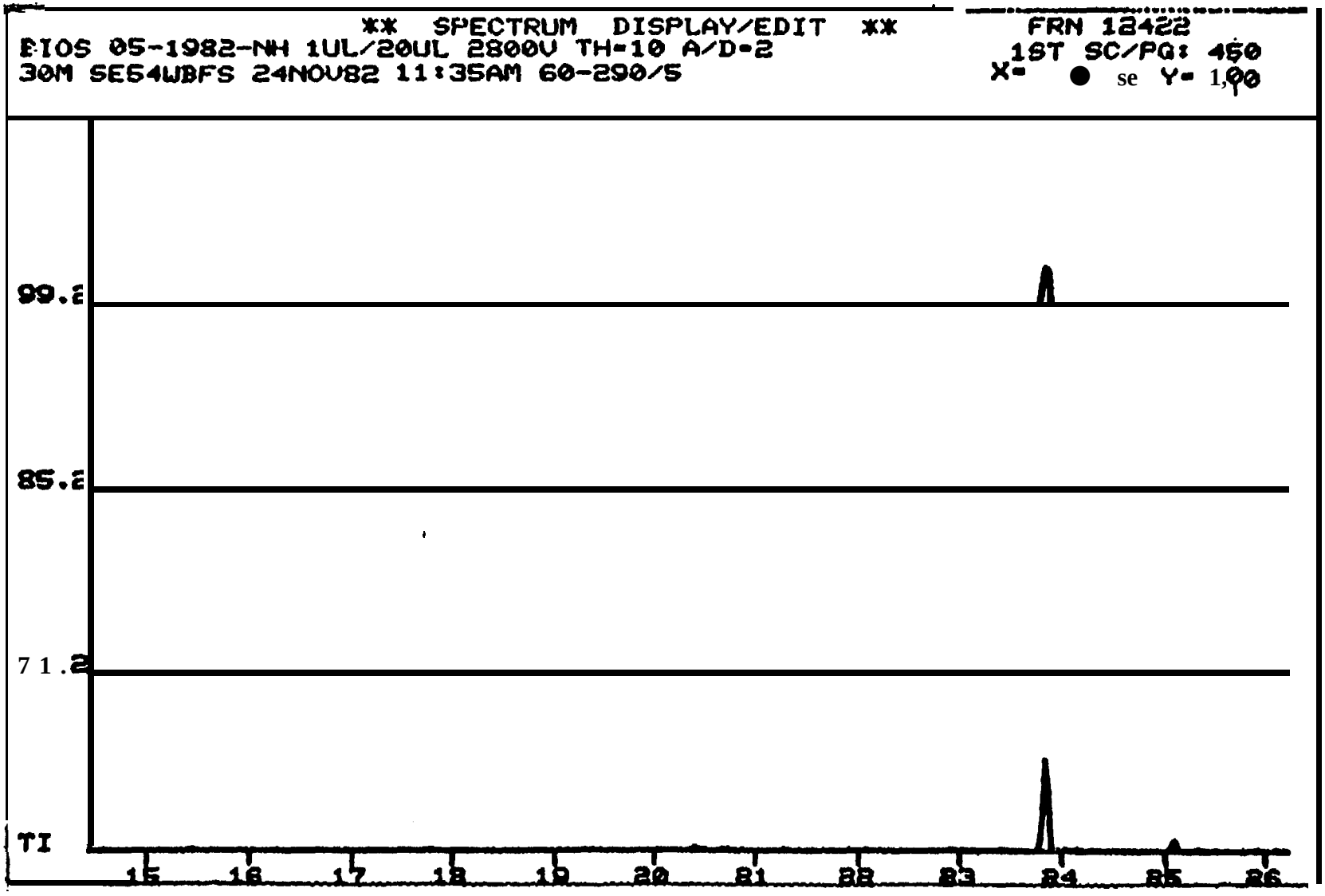
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X= se Y= 1,00
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85.2
71.2
TI
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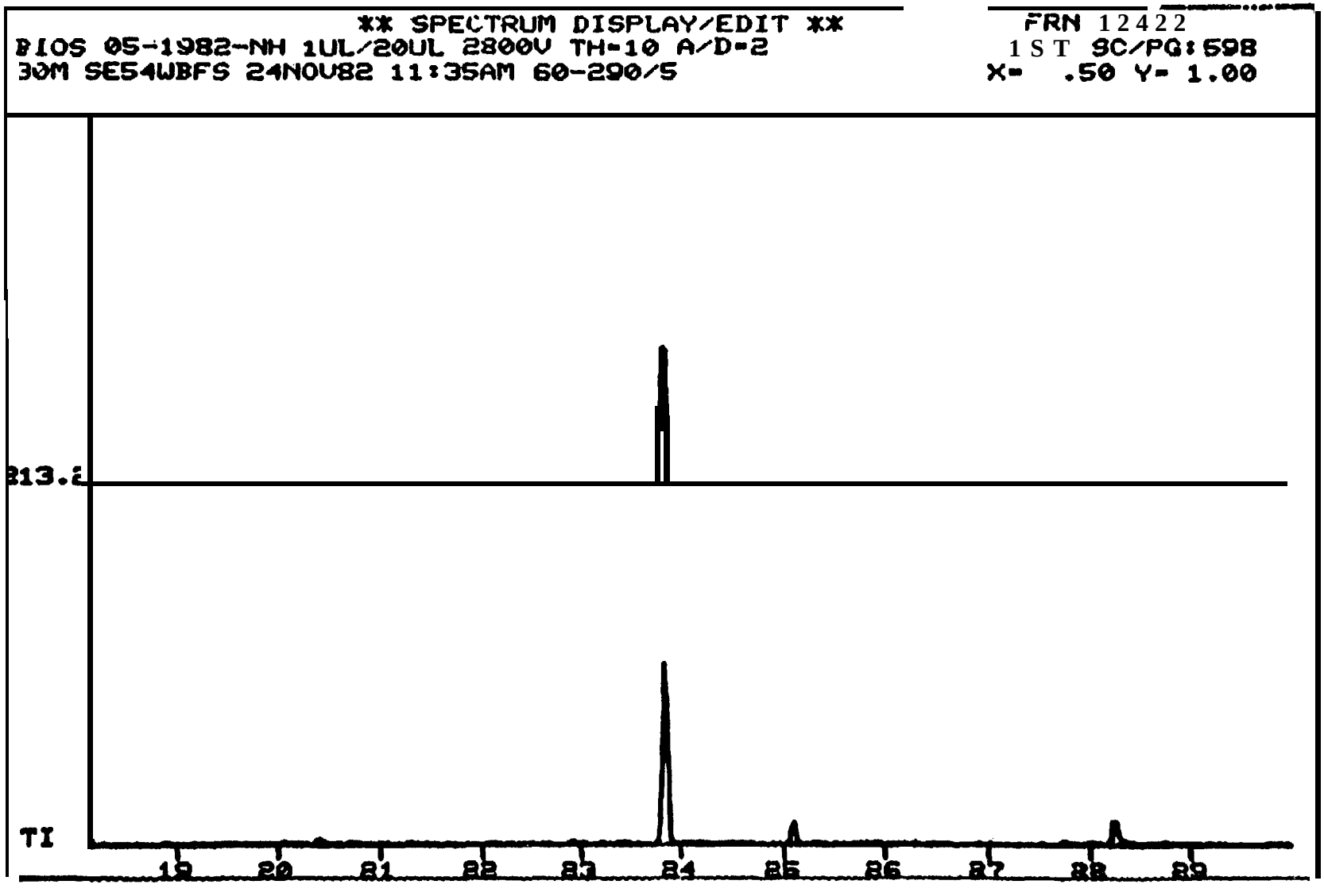
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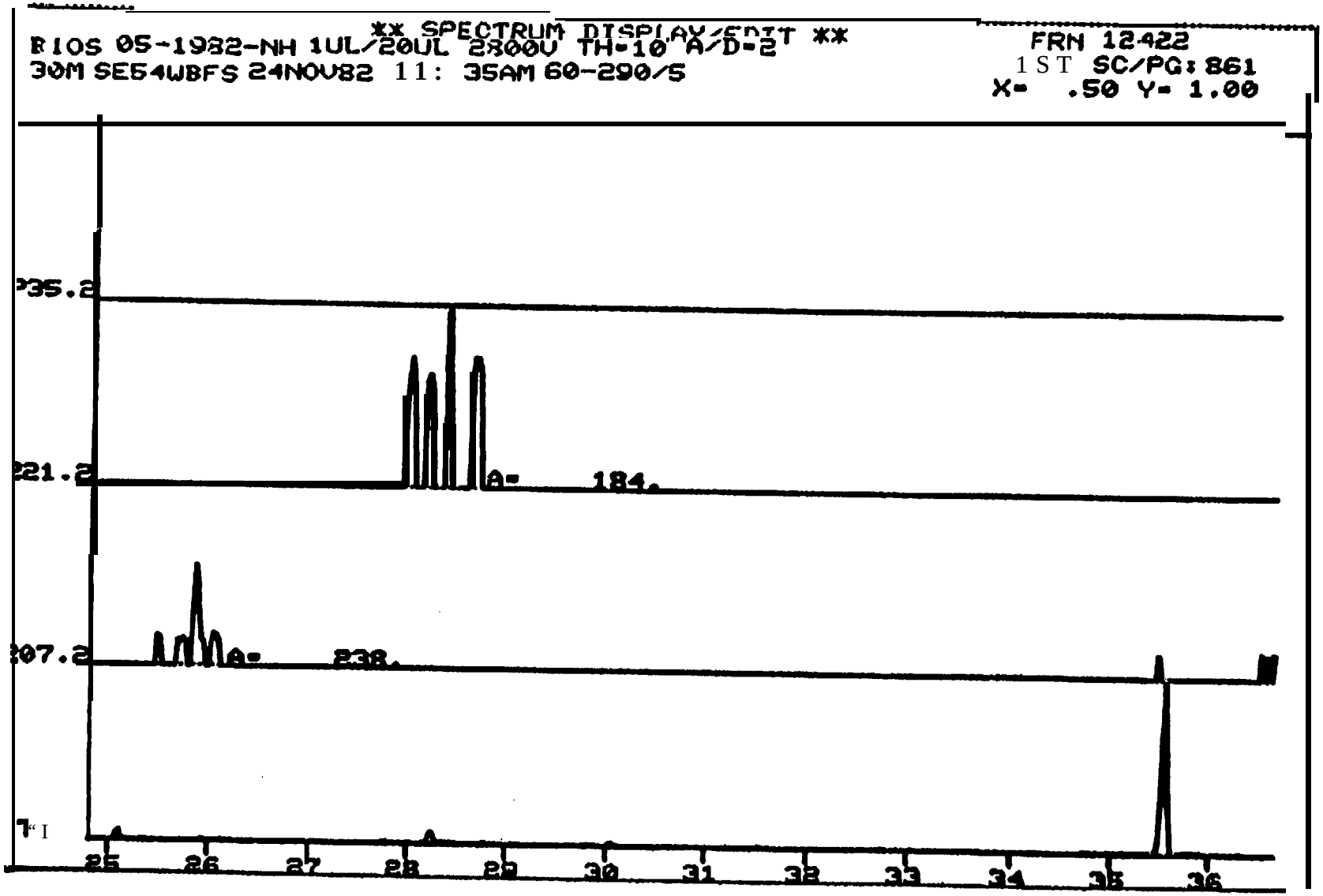
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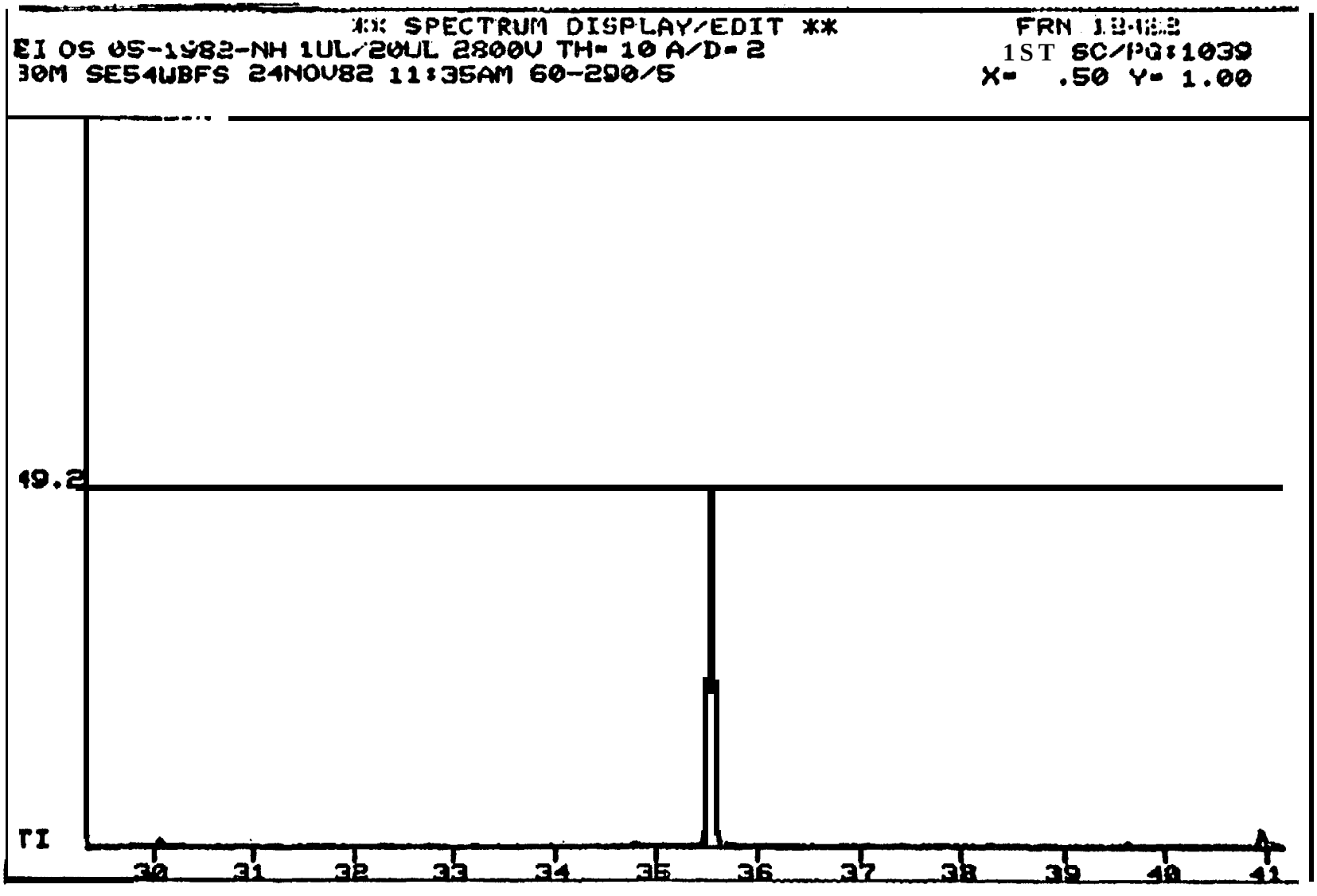
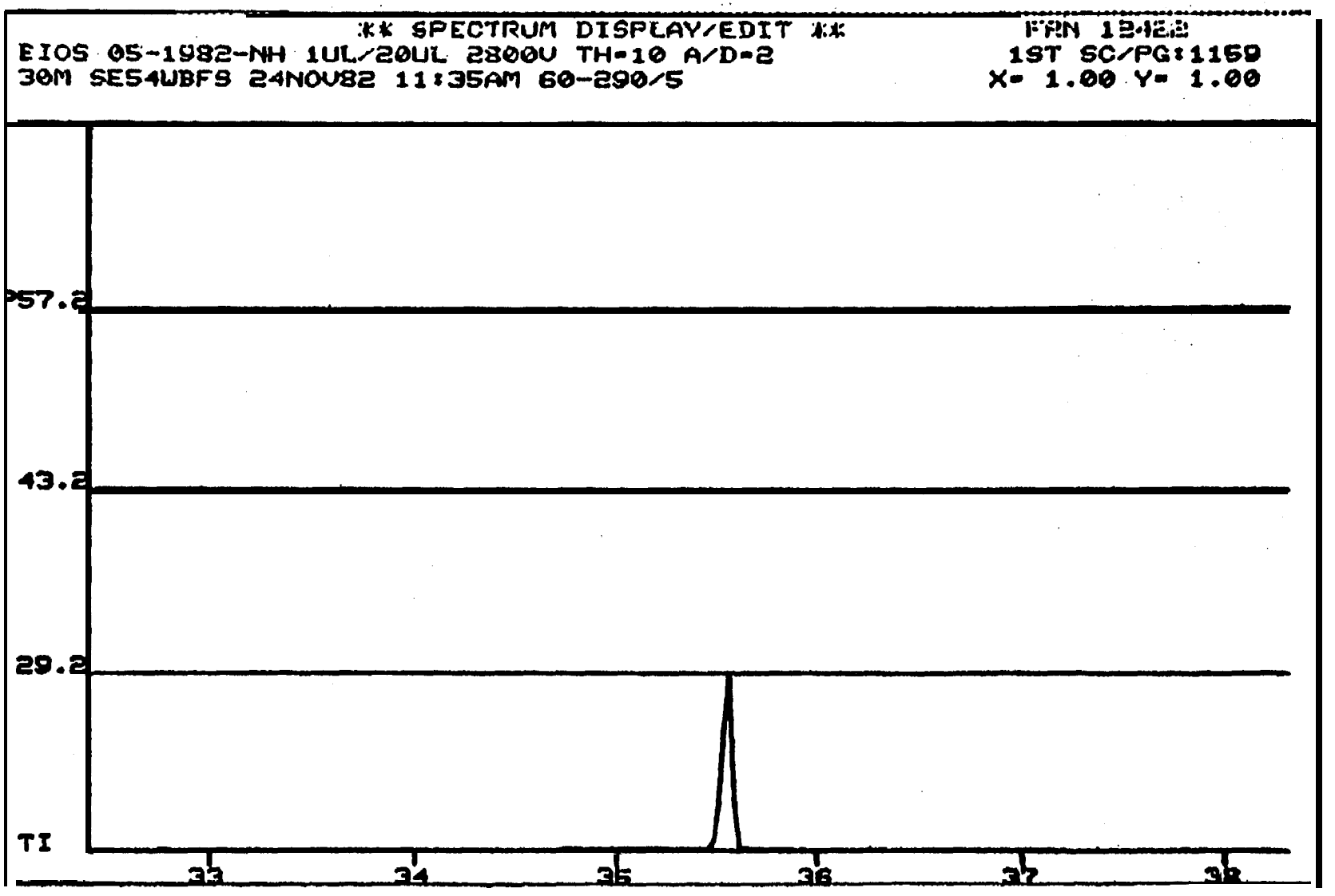
BIOS 05-1982-NH 1UL/20UL 2800V TH=10 A/D=2
30M SE54WBFS 24NOV82 11: 35AM 60-290/5
FRN 12422
1ST SC/PG: 861
X= .50 Y= 1.00
235.2
221.2
A= 184.
207.2
A= 238.
25
26
27
28
29
30
31
32
33
34
35
36


** SPECTRUM DISPLAY/EDIT **
FRN 12-182
EI OS 05-1982-NH 1UL/20UL 2800V TH= 10 A/D= 2
1ST SC/PG:1039
30M SE54WBFS 24NOV82 11:35AM 60-290/5
X= .50 Y= 1.00
49.2
TI
30
31
32
33
34
35
36
37
38
39
40
41


** SPECTRUM DISPLAY/EDIT **
FRN 13:42:2
EIOS 05-1982-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PG:1159
30M SE54UBFS 24NOV82 11:35AM 60-290/5
X= 1.00 Y= 1.00
57.2
43.2
29.2
TI
33
34
35
36
37
38

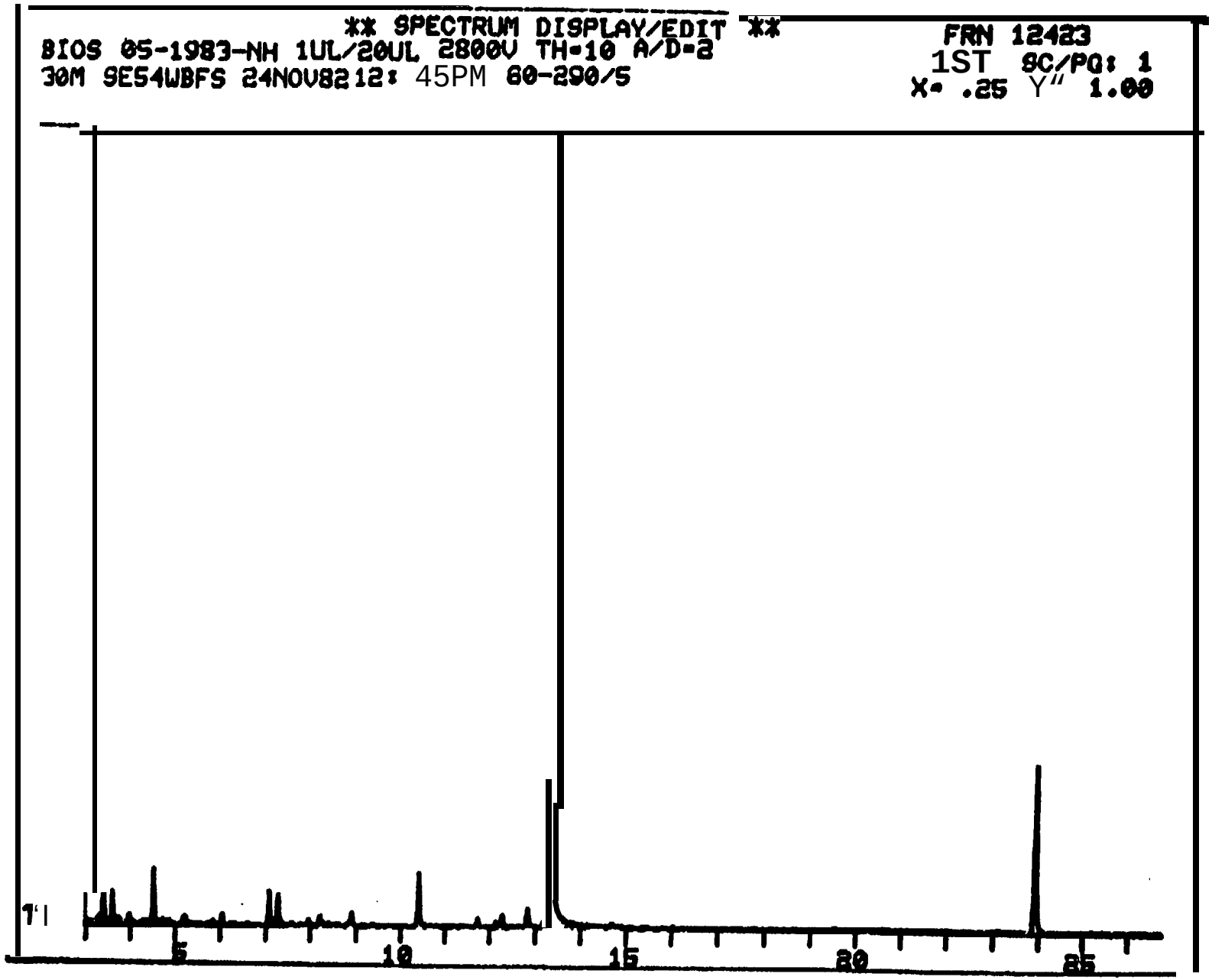
FILE NUMBER 12423

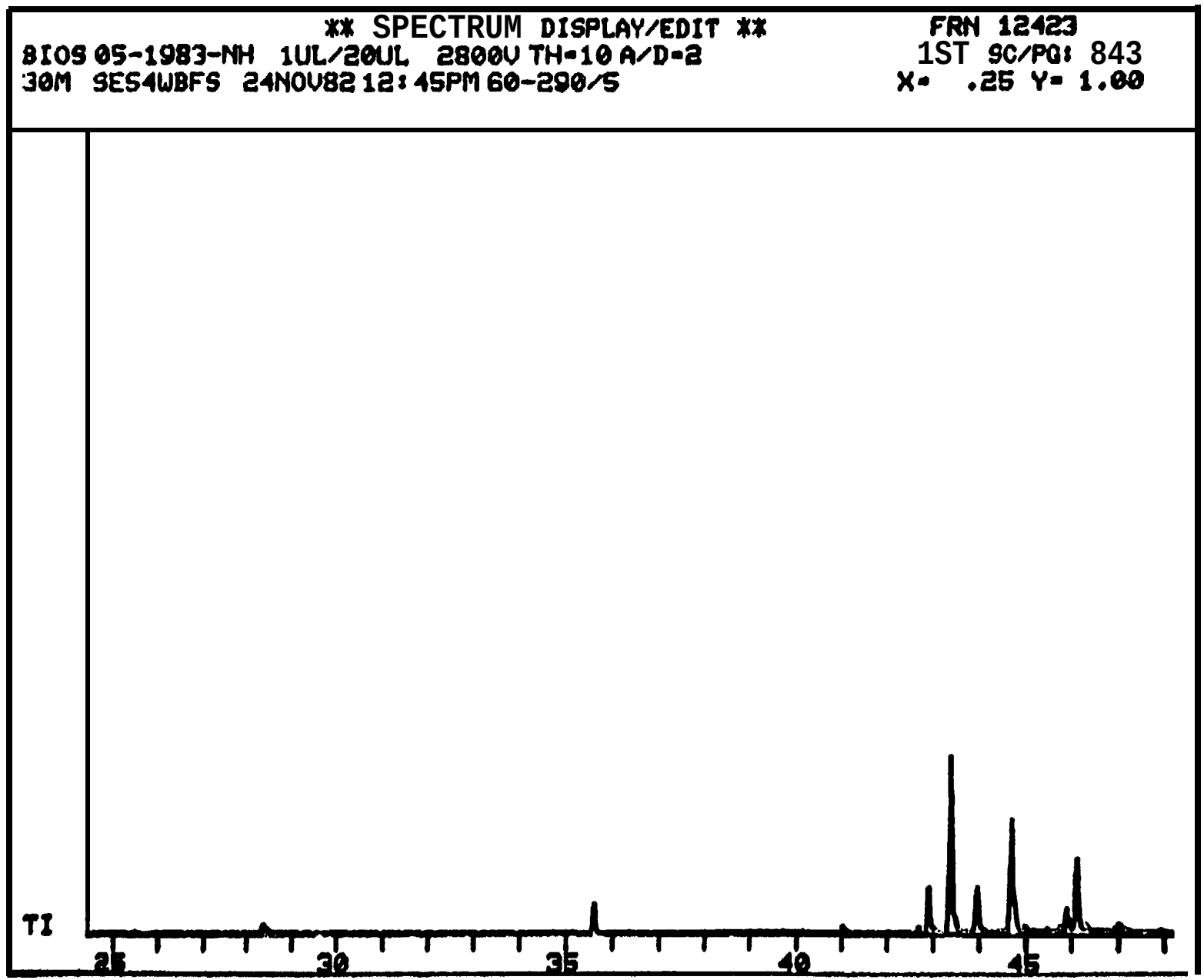
ENTRY	TIME	MASS	AREA	%
1	25.9	207.2	238.	56.40
2	28.4	221.2	184.	43.60

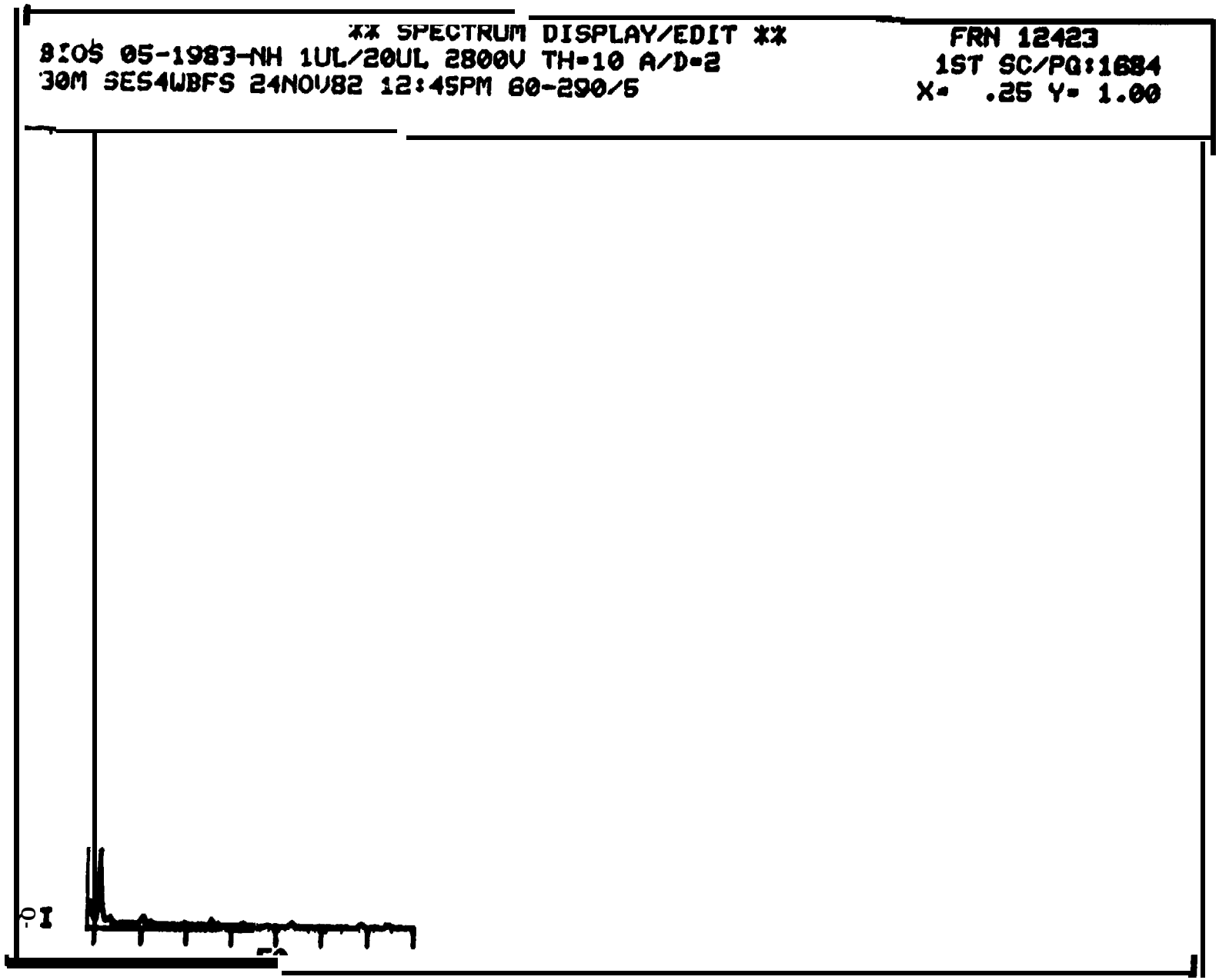
CAL % ON ENTRY?

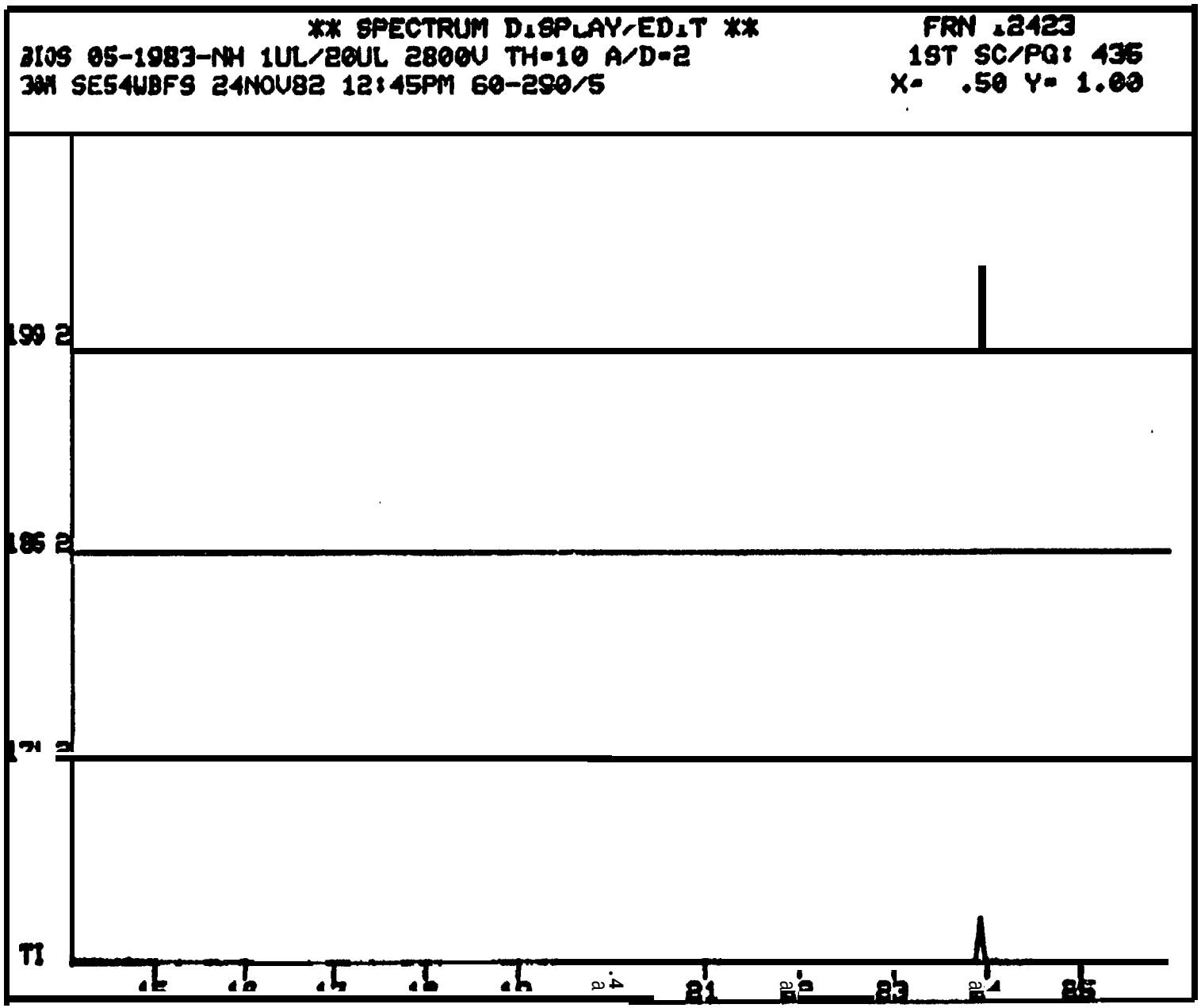
APPENDIX III :

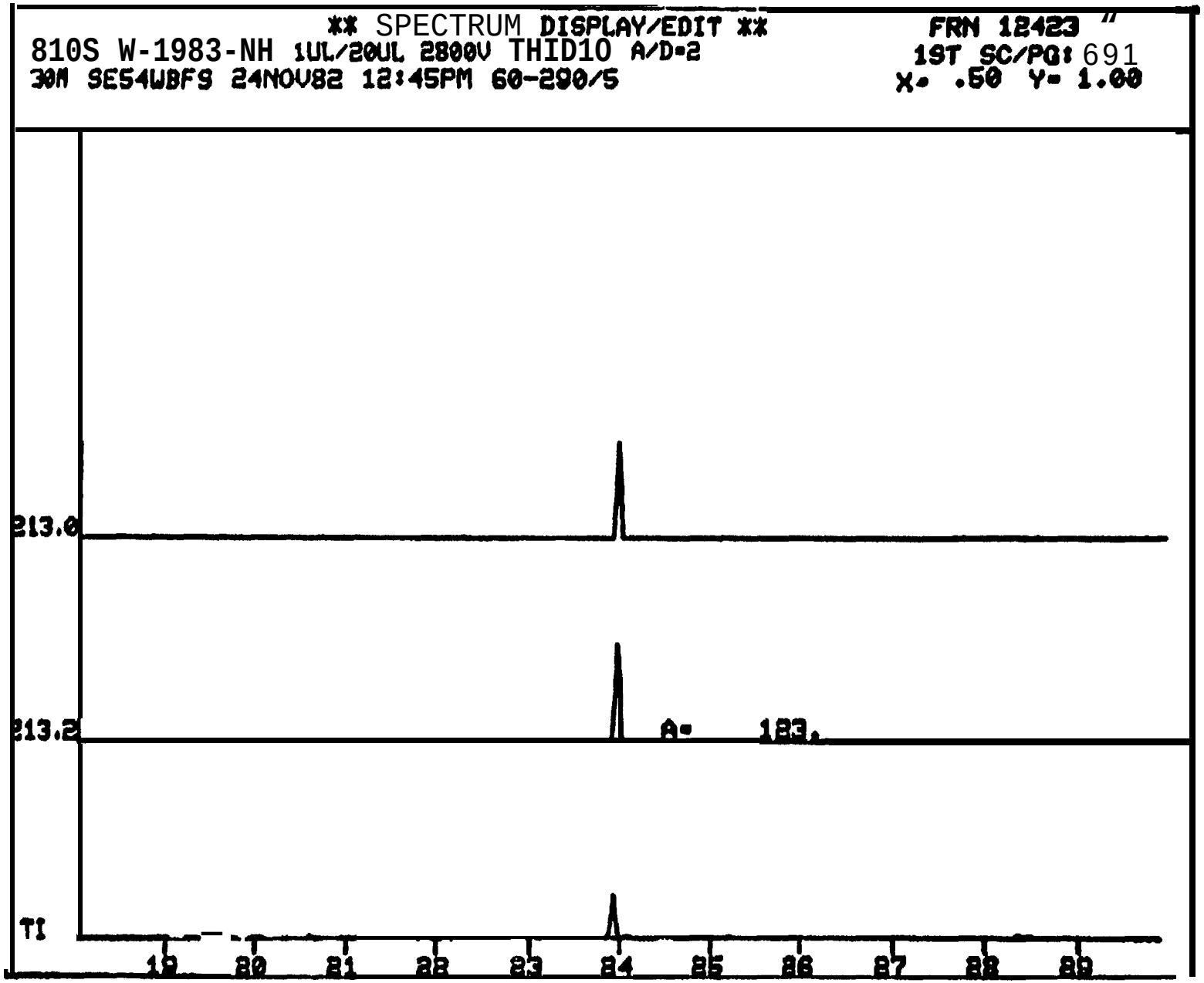
ASTARTE BOREALIS: BAY 9: 2 WEEKS

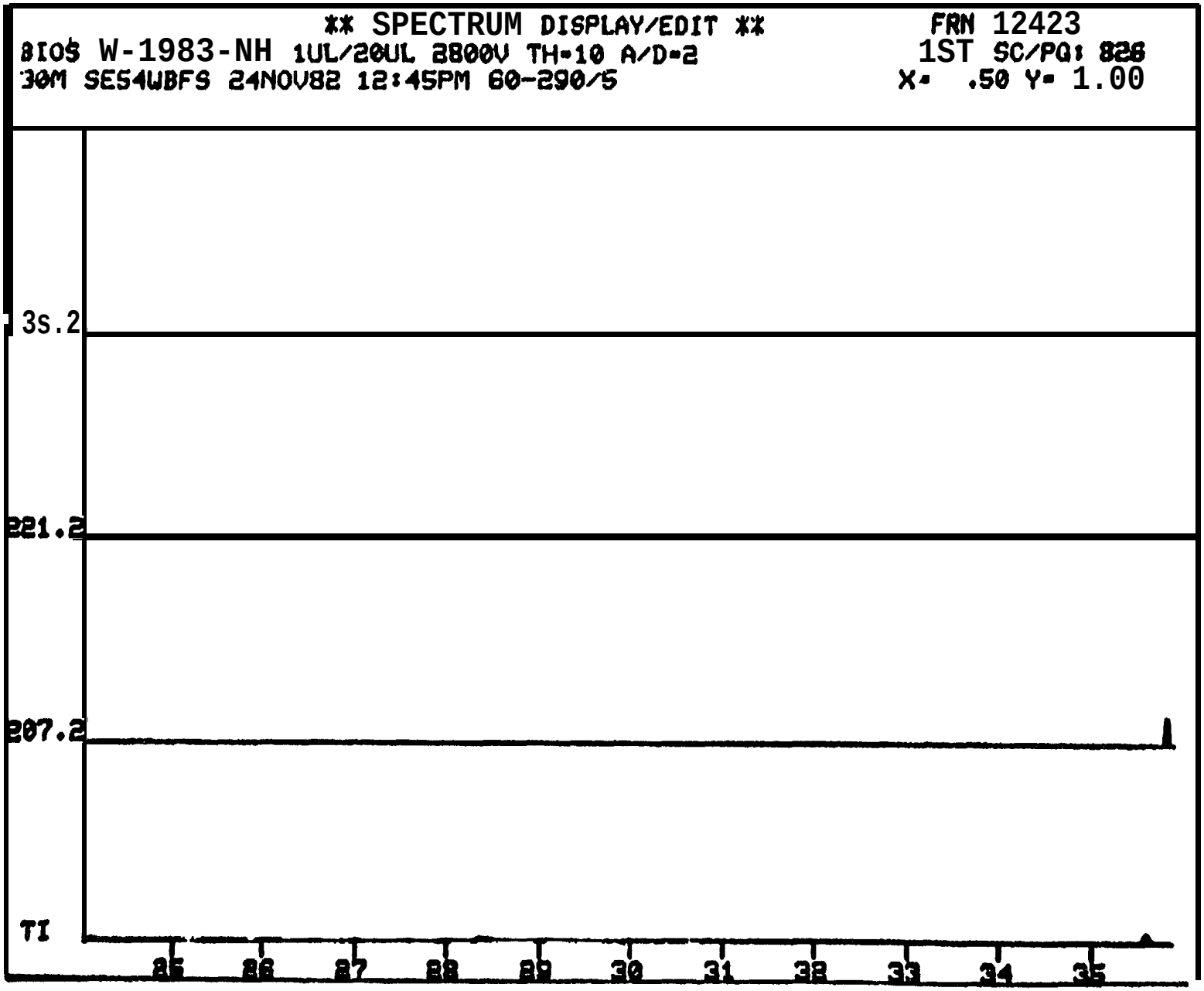

** SPECTRUM DISPLAY/EDIT **
BIOS 05-1983-NH 1UL/20UL 2800V TH=10 A/D=2
30M SE54WBFS 24NOV82 12: 45PM 80-290/5
FRN 12423
1ST SC/PG: 1
X= .25 Y= 1.00
T1
5
10
15
20
25

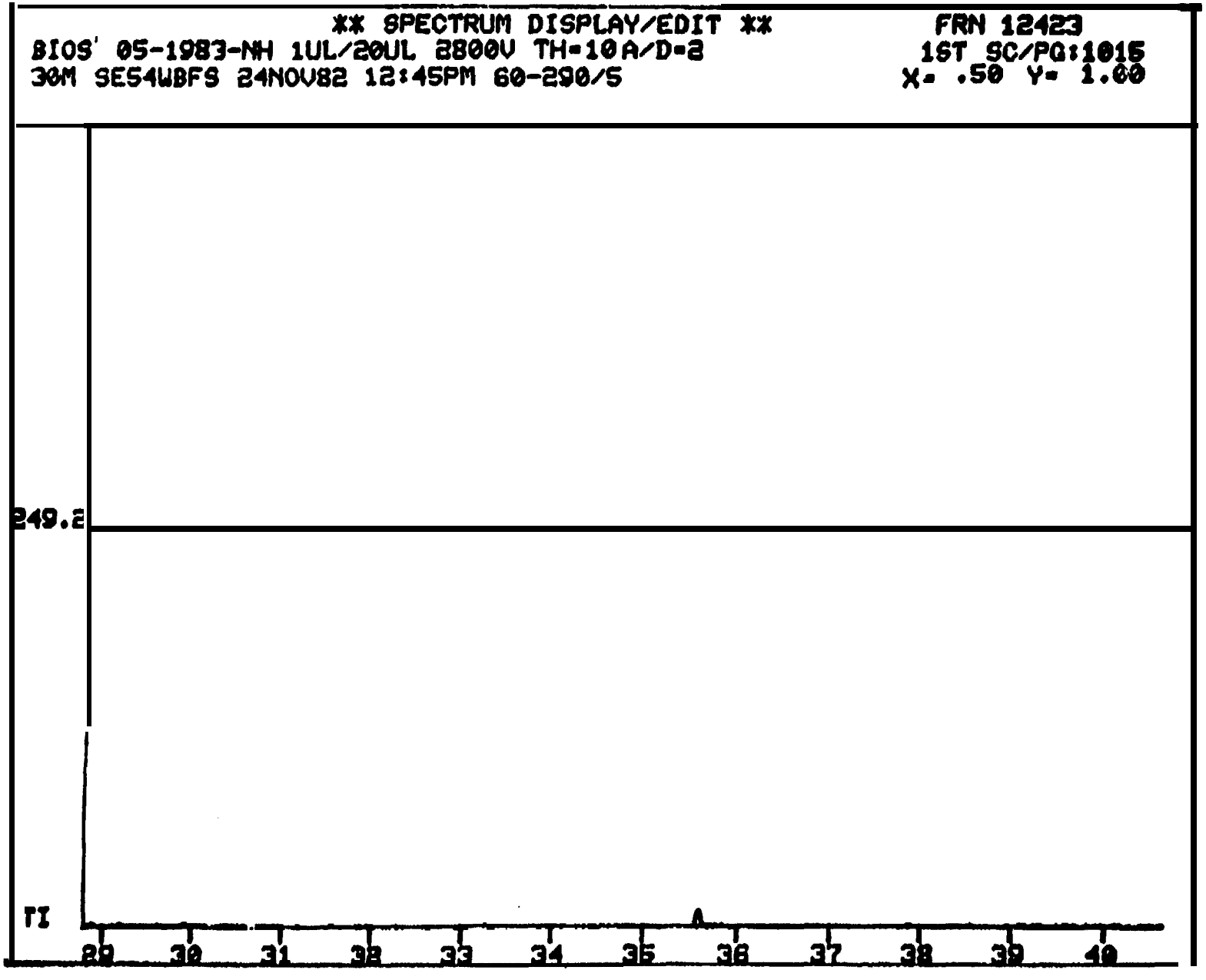

** SPECTRUM DISPLAY/EDIT **
FRN 12423
BIOS 05-1983-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PG: 843
30M SES4WBFS 24NOV82 12:45PM 60-290/5
X= .25 Y= 1.00
TI
25
30
35
40
45

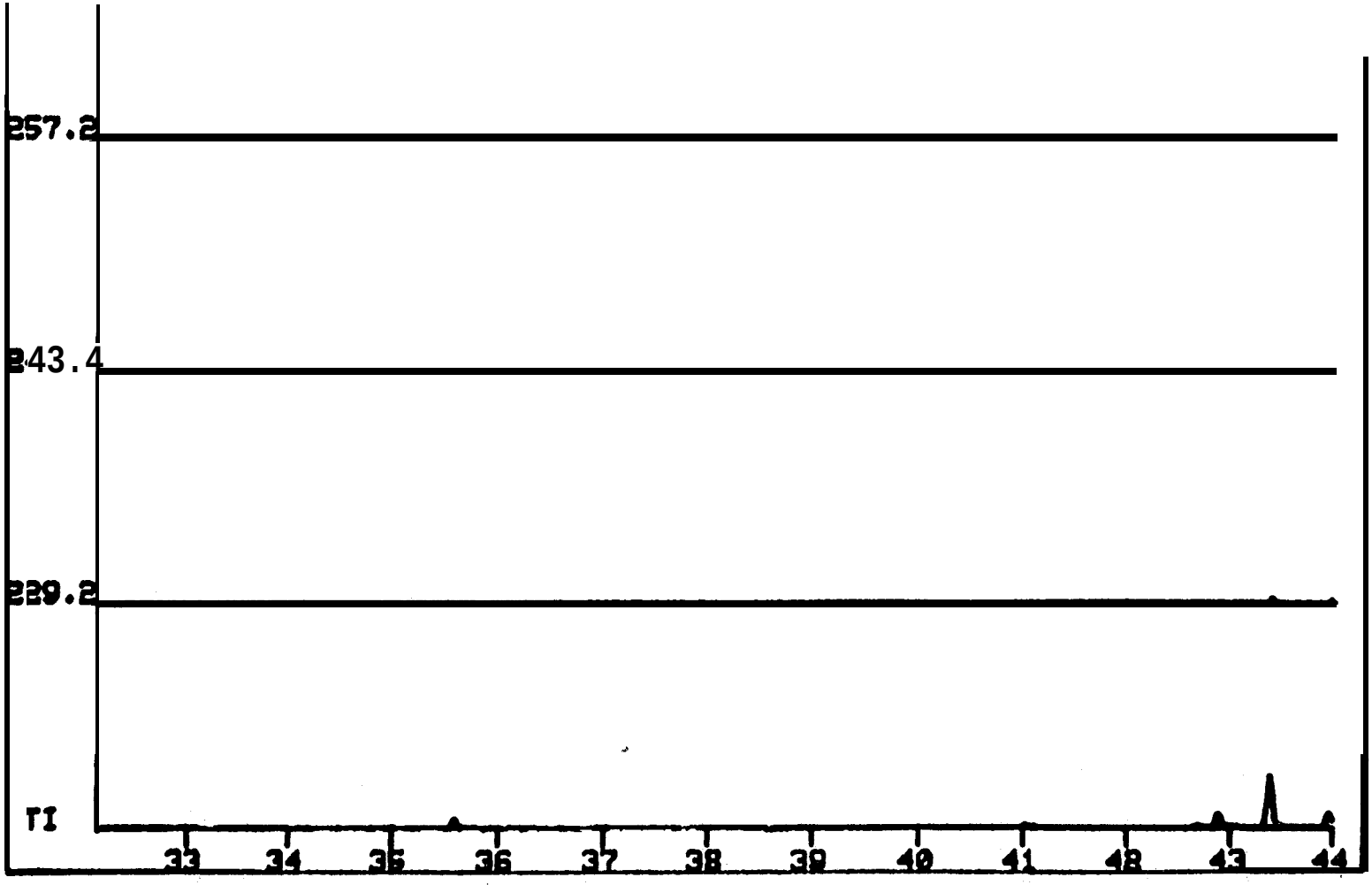

** SPECTRUM DISPLAY/EDIT **
FRN 12423
BIOS 05-1983-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PQ:1684
30M SES4WBFS 24NOV82 12:45PM 60-290/5
X= .25 Y= 1.00
Q-
I


** SPECTRUM DISPLAY/EDIT **
FRN 2423
BIOS 05-1983-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PQ: 436
3M SE54WBFS 24NOV82 12:45PM 60-290/5
X= .50 Y= 1.00
199
186
171
TI


** SPECTRUM DISPLAY/EDIT **
FRN 12423
810S W-1983-NH 1UL/20UL 2800V THID10 A/D=2
19T SC/PG: 691
30M SE54WBFS 24NOV82 12:45PM 60-290/5
X= .50 Y= 1.00
213.0
213.2
A= 183.
TI
19
20
21
22
23
24
25
26
27
28
29


** SPECTRUM DISPLAY/EDIT **
FRN 12423
BIOS W-1983-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PQ: 826
30M SES4WBFS 24NOV82 12:45PM 60-290/5
X= .50 Y= 1.00
3s.2
221.2
207.2
TI
85
86
87
88
89
30
31
32
33
34
35


** SPECTRUM DISPLAY/EDIT **
FRN 12423
BIOS' 05-1983-NH 1UL/20UL 2800V TH=10A/D=2
1ST SC/PQ:1015
30M SE54WBFS 24NOV82 12:45PM 60-290/5
X= .50 Y= 1.00
249.2
TI
29
30
31
32
33
34
35
36
37
38
39
40



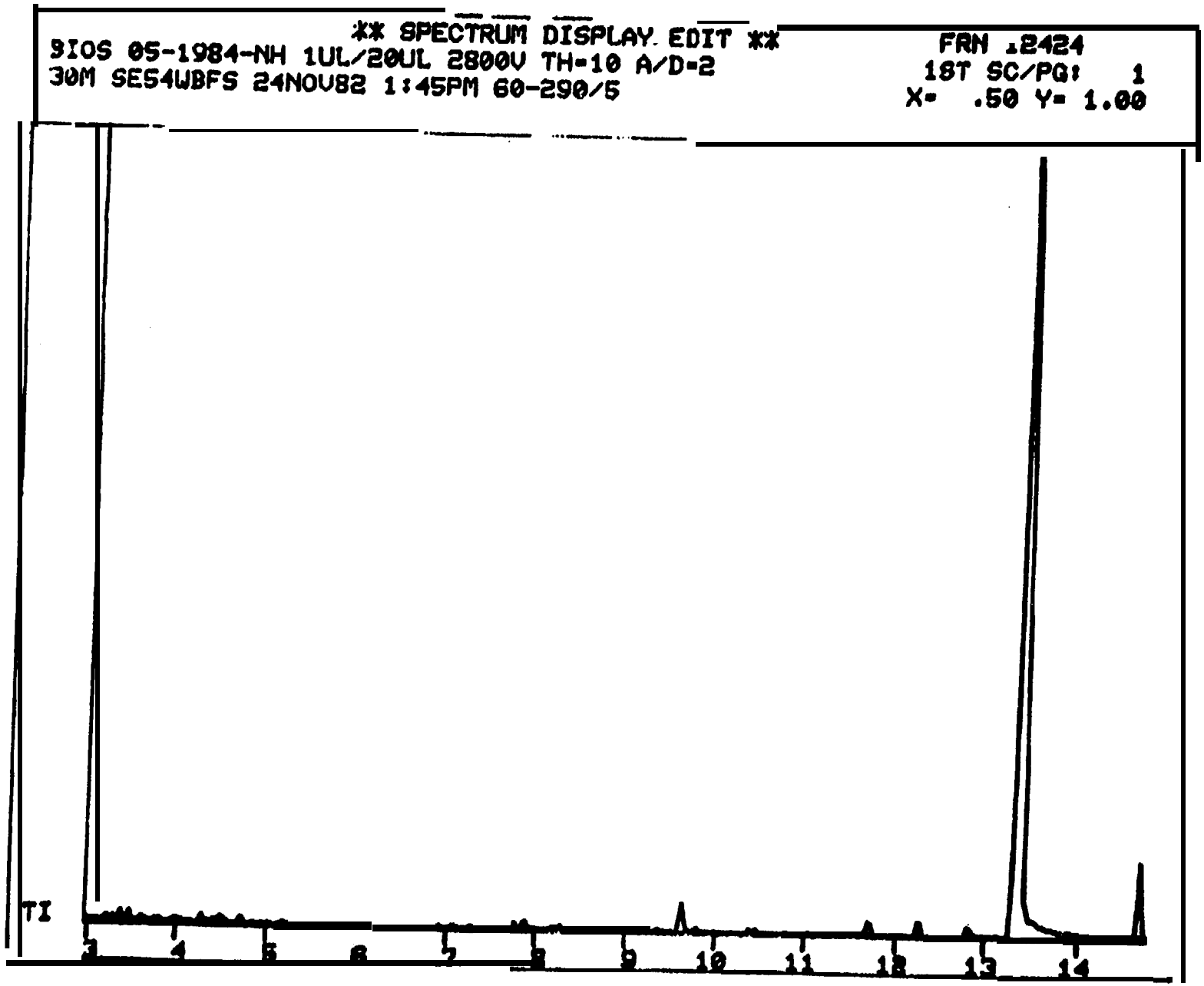
FILE NUMBER 1%423

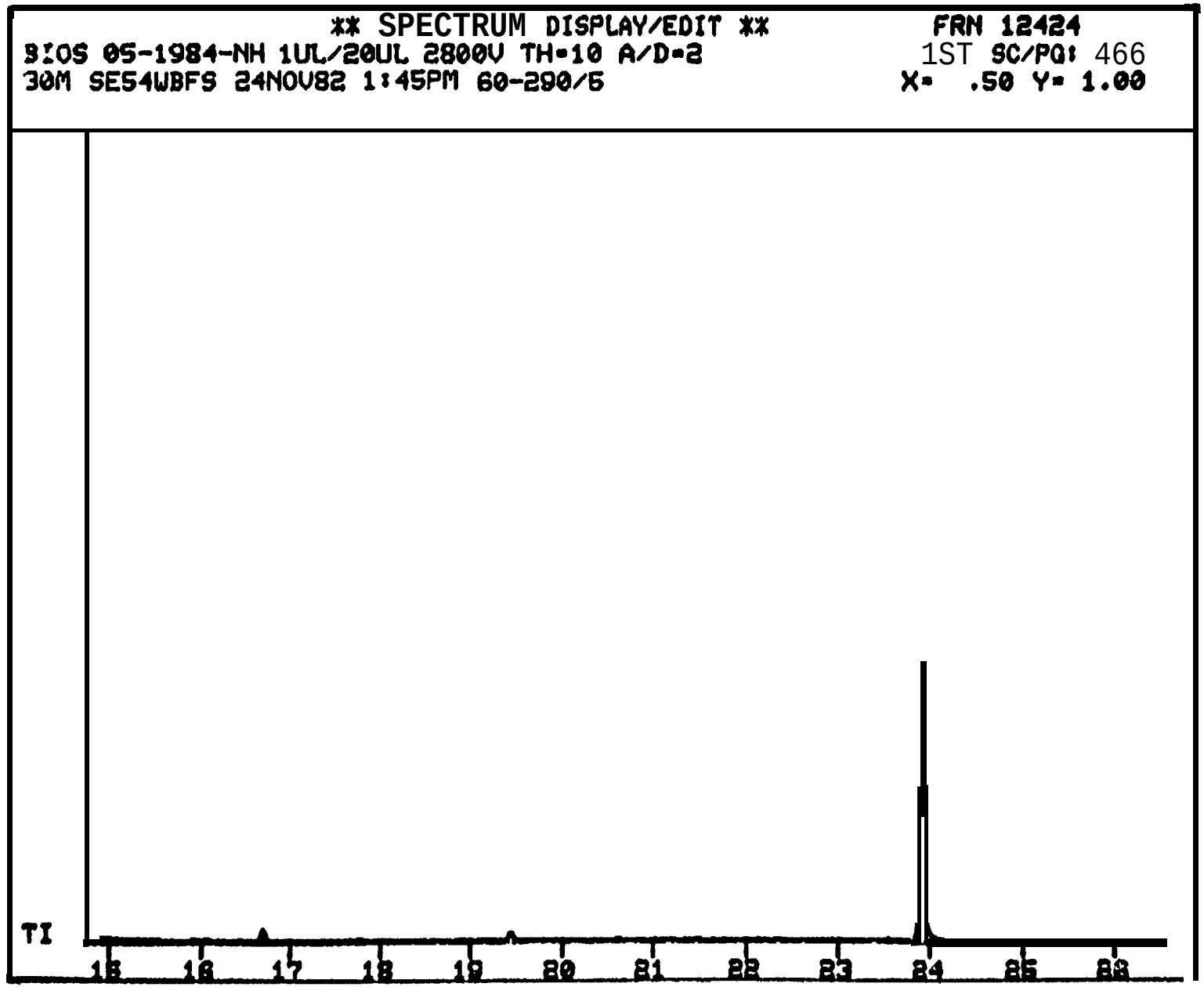
ENTRY	TIME	MASS	AREA	%
1	23.9	213.2	123.	100.00

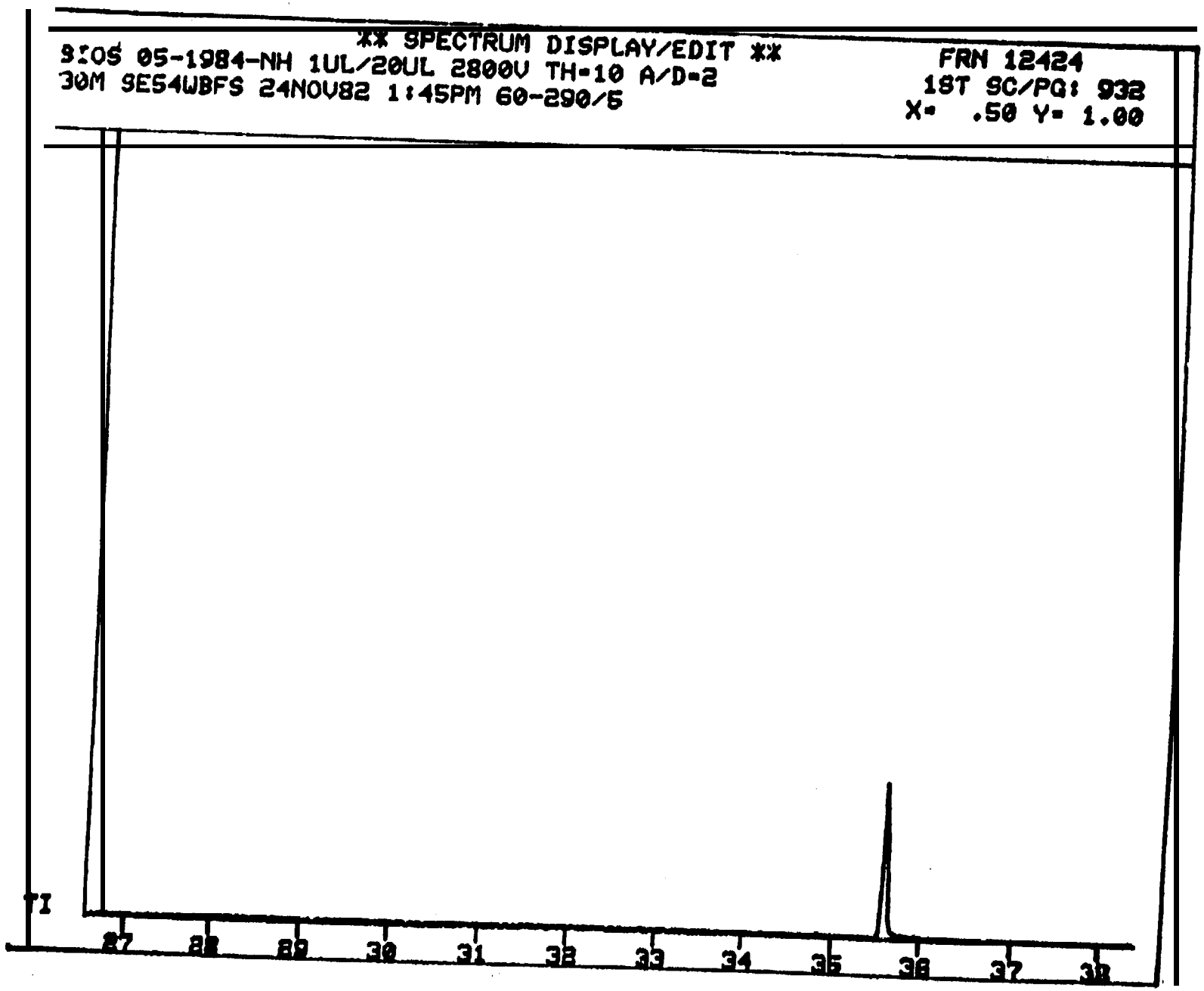
CAL % ON ENTRY?

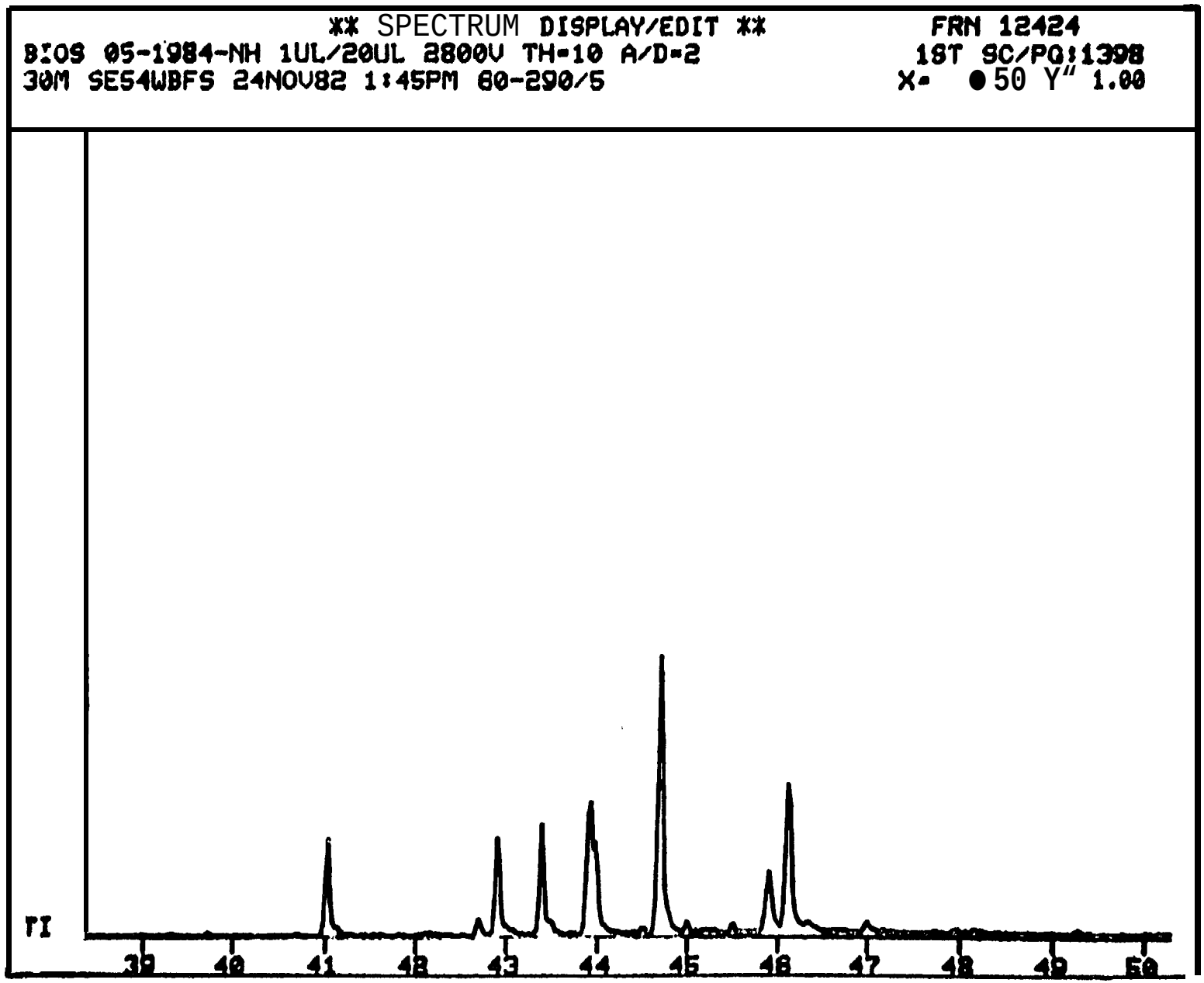
APPENDIX IV:

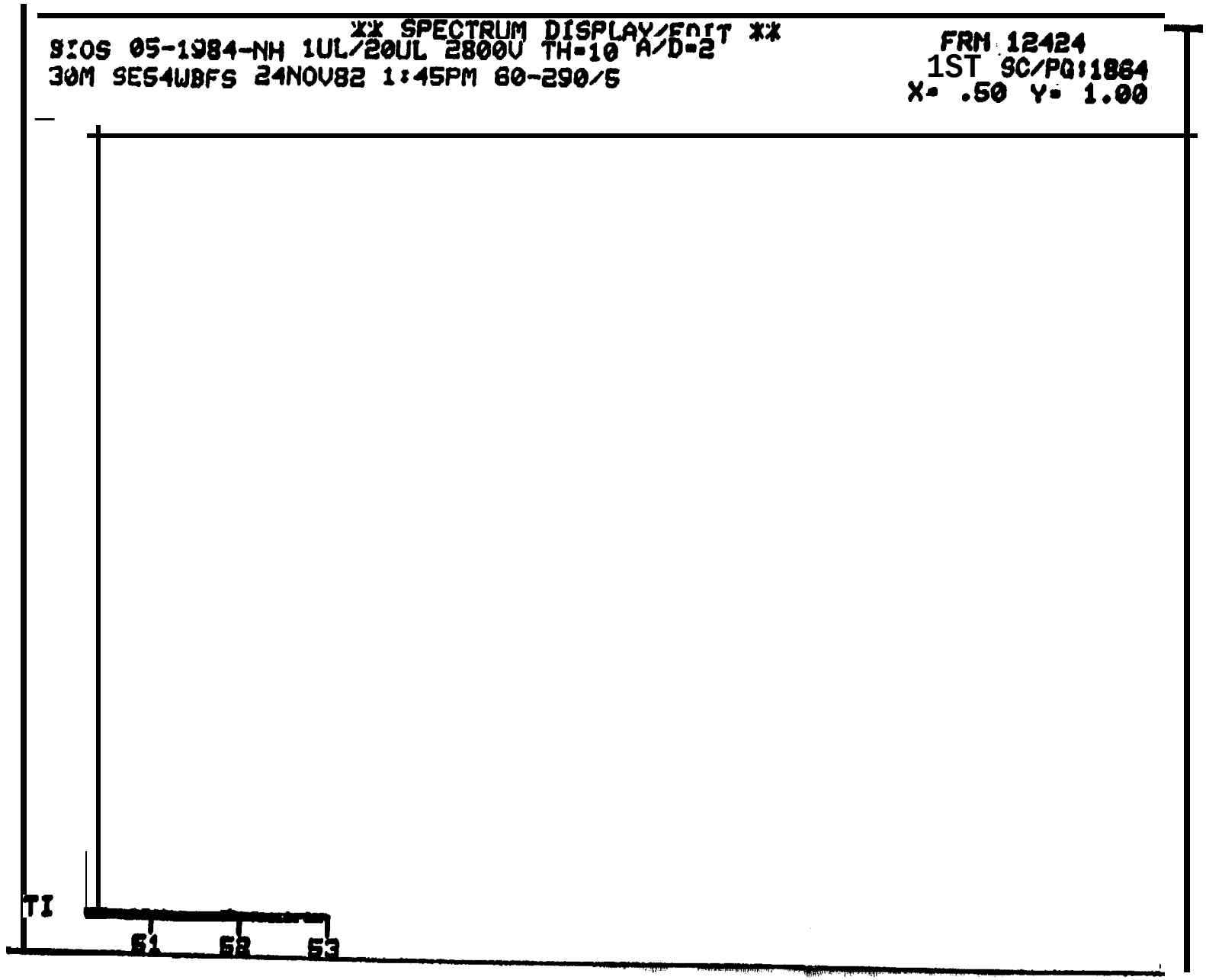
SERRIPES GROENLANDICUS: BAY 10: 1 DAY

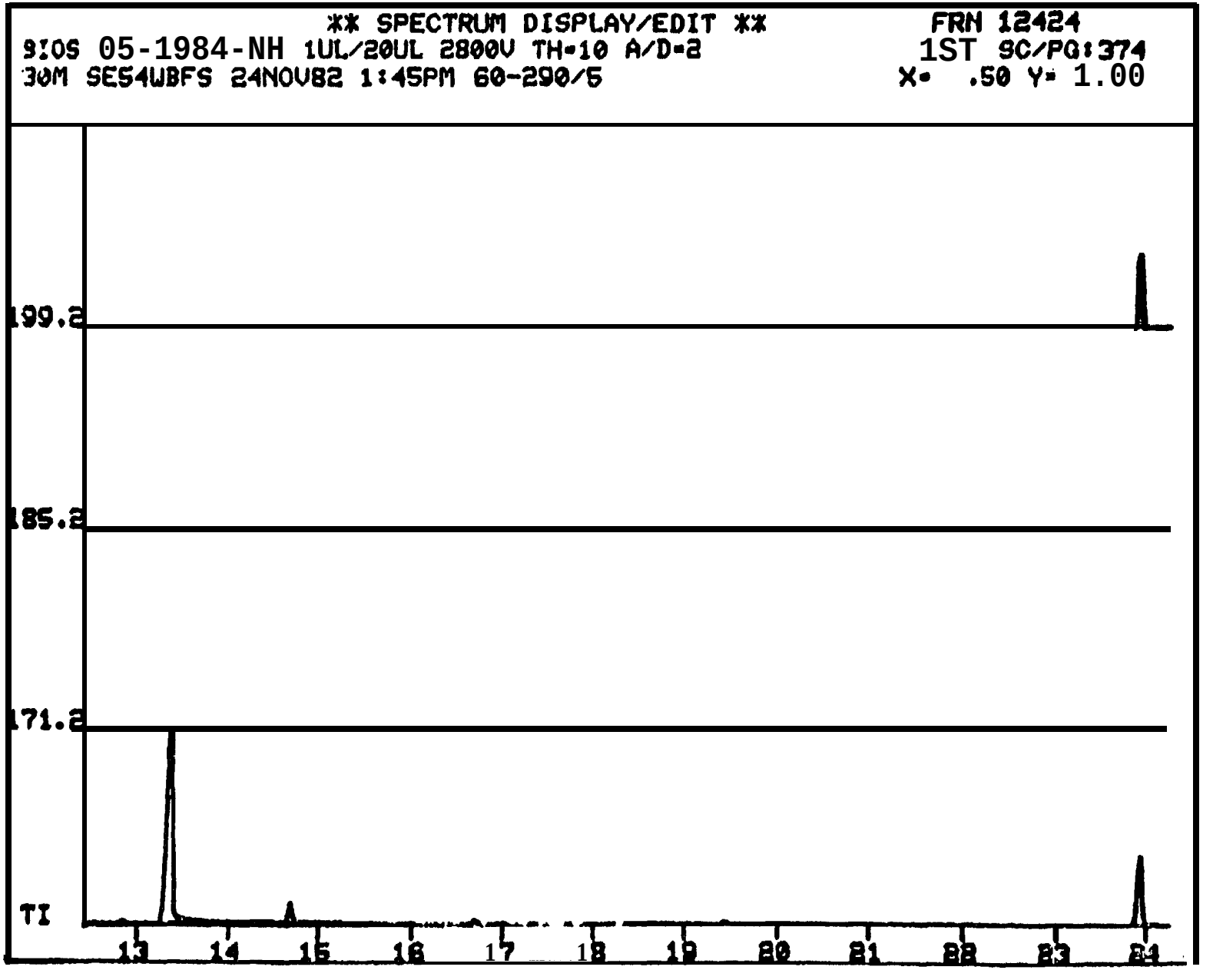

** SPECTRUM DISPLAY EDIT **
FRN .2424
9IOS 05-1984-NH 1UL/20UL 2800V TH-10 A/D-2
1ST SC/PG: 1
30M SE54WBFS 24NOV82 1:45PM 60-290/5
X= .50 Y= 1.00
TI
3
4
5
6
7
8
9
10
11
12
13
14

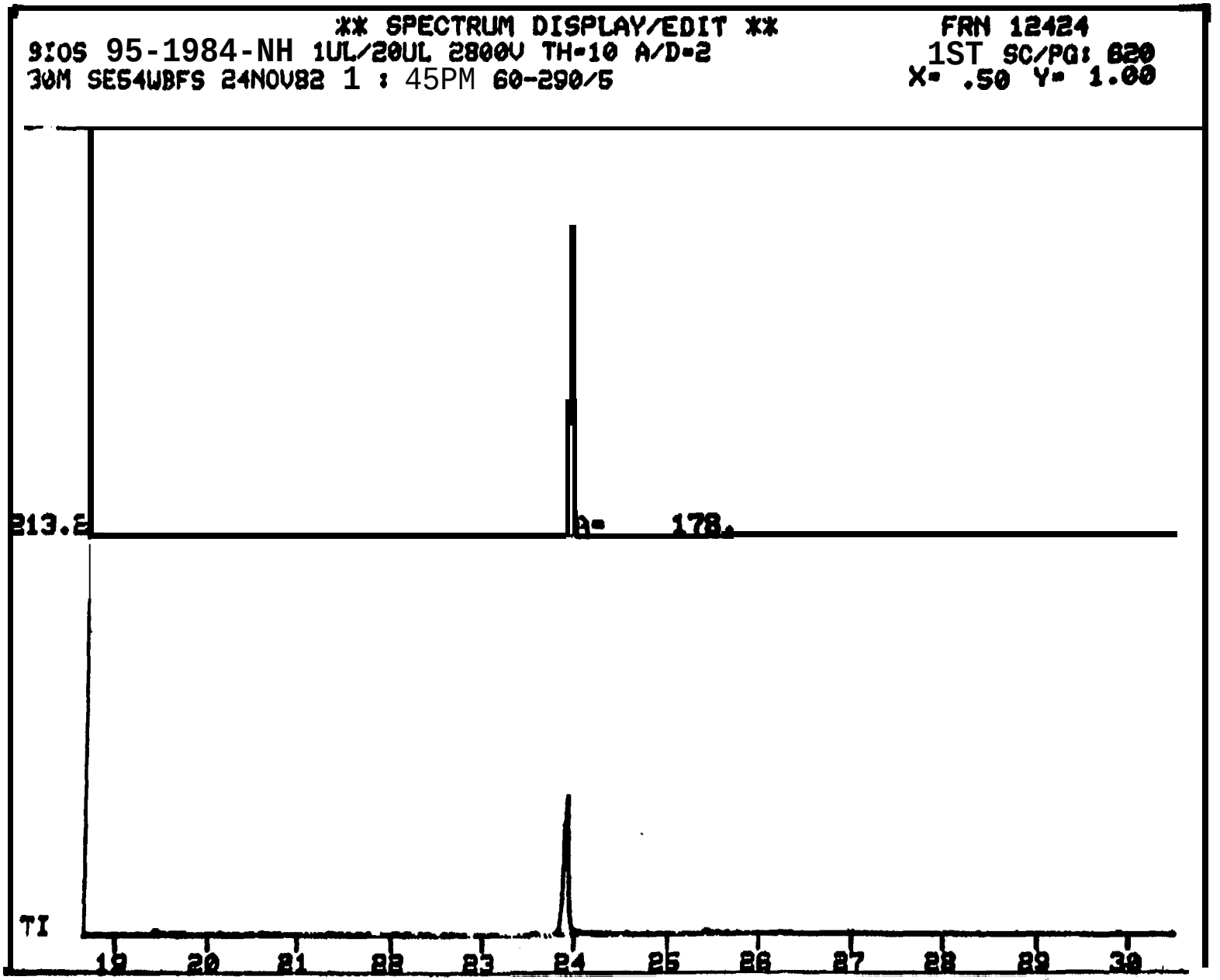

** SPECTRUM DISPLAY/EDIT **
FRN 12424
BIOS 05-1984-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PQ: 466
30M SE54WBFS 24NOV82 1:45PM 60-290/5
X= .50 Y= 1.00
TI
15
16
17
18
19
20
21
22
23
24
25
26

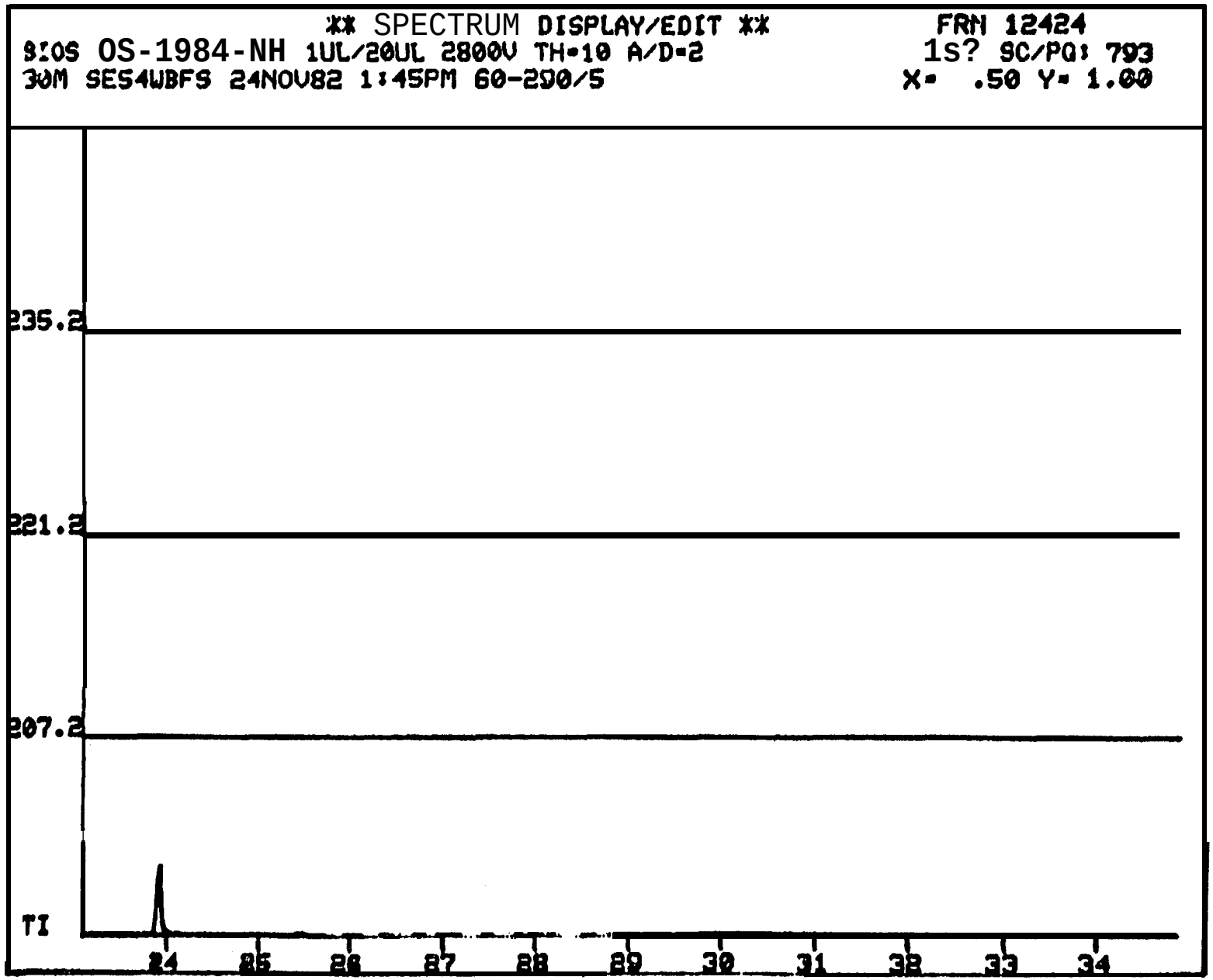

** SPECTRUM DISPLAY/EDIT **
9:05 05-1984-NH 1UL/20UL 2800V TH=10 A/D=2
30M 9E54WBFS 24NOV82 1:45PM 60-290/5
FRN 12424
1ST SC/PQ: 932
X= .50 Y= 1.00
TI
27
28
29
30
31
32
33
34
35
36
37
38

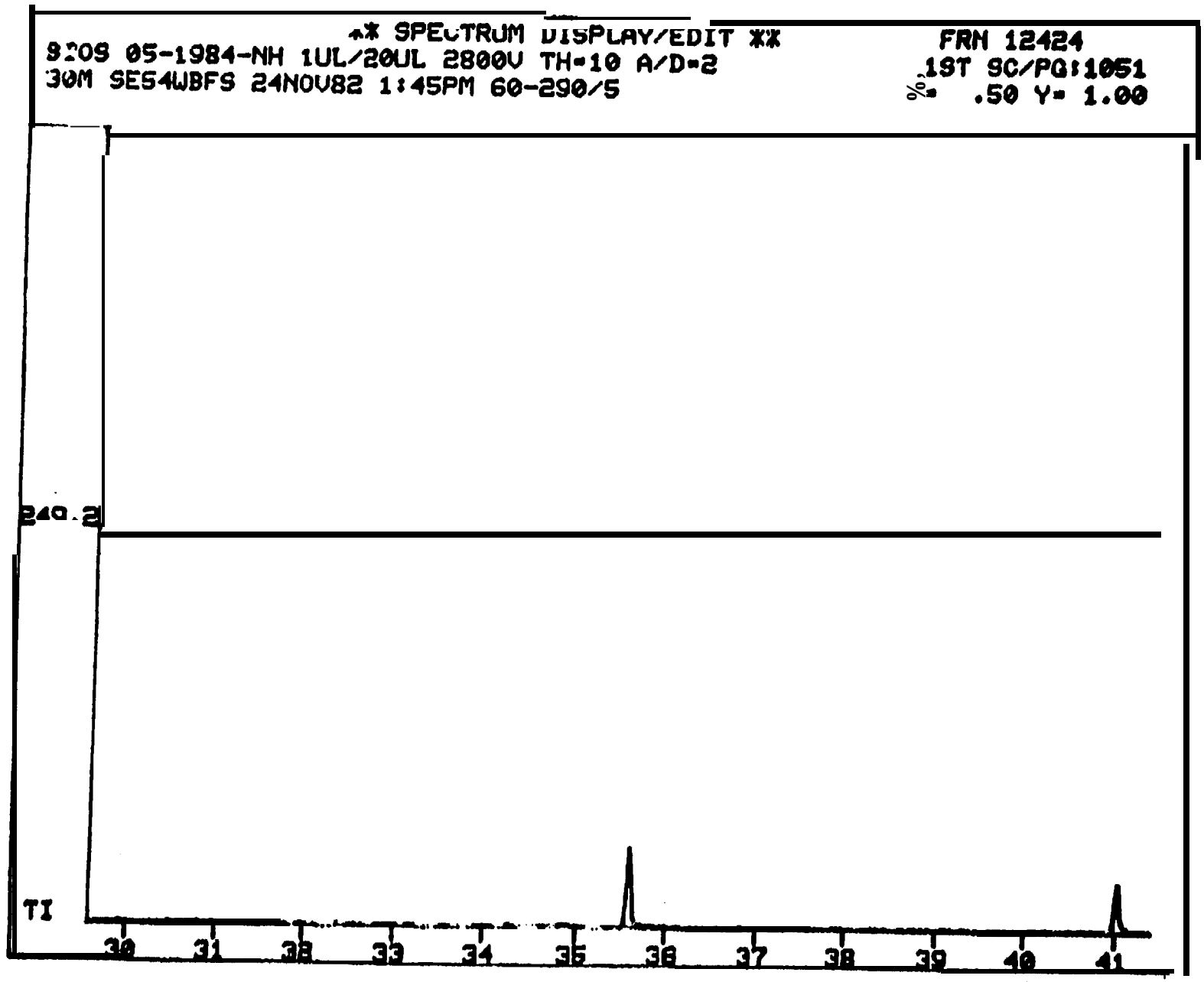

** SPECTRUM DISPLAY/EDIT **
FRN 12424
B:OS 05-1984-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PQ:1398
30M SE54WBFS 24NOV82 1:45PM 80-290/5
X= ●50 Y" 1.00
TI
39
40
41
42
43
44
45
46
47
48
49
50


** SPECTRUM DISPLAY/EDIT **
SIOS 05-1984-NH 1UL/20UL 2800V TH=10 A/D=2
30M SE54WBFS 24NOV82 1:45PM 60-290/5
FRM 12424
1ST SC/PQ:1864
X= .50 Y= 1.00
TI
61
62
63


** SPECTRUM DISPLAY/EDIT **
FRN 12424
9:05 05-1984-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PG:374
30M SE54WBFS 24NOV82 1:45PM 60-290/5
X= .50 Y= 1.00
199.2
185.2
171.2
TI
13
14
15
16
17
18
19
20
21
22
23
24

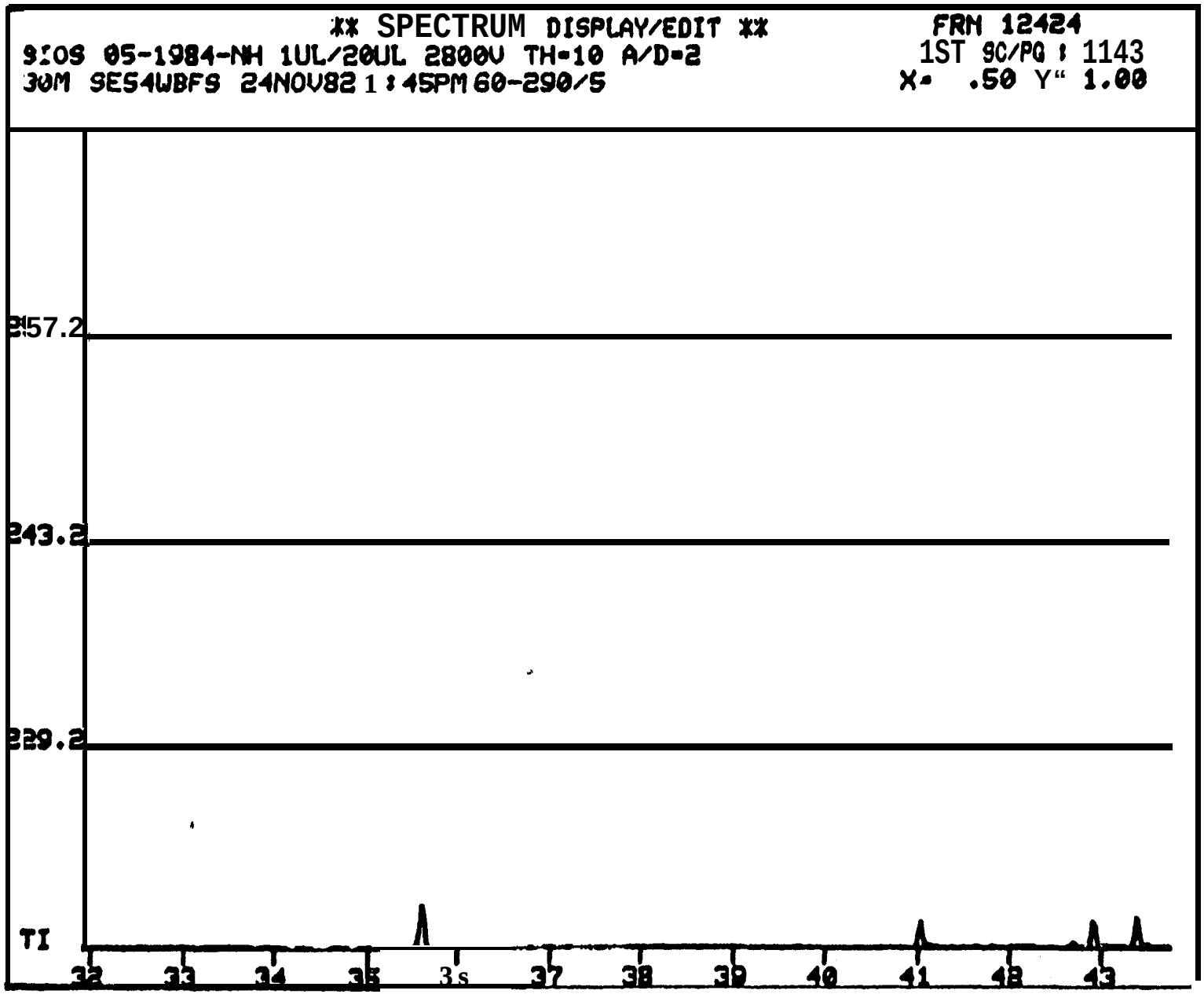

** SPECTRUM DISPLAY/EDIT **
FRN 12424
9:05 95-1984-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PQ: 820
30M SE54WBFS 24NOV82 1 : 45PM 60-290/5
X= .50 Y= 1.00
213.2
178.
TI
19
20
21
22
23
24
25
26
27
28
29
30


** SPECTRUM DISPLAY/EDIT **
FRN 12424
SIOS OS-1984-NH 1UL/20UL 2800V TH=10 A/D=2
1S? SC/PQ: 793
30M SES4WBFS 24NOV82 1:45PM 60-290/5
X= .50 Y= 1.00
235.2
221.2
207.2
TI
24
25
26
27
28
29
30
31
32
33
34


** SPECTRUM DISPLAY/EDIT **
9209 05-1984-NH 1UL/20UL 2800V TH=10 A/D=2
30M SE54WBFS 24NOV82 1:45PM 60-290/5
FRN 12424
1ST SC/PG:1051
%= .50 Y= 1.00
240.2
TI
30
31
32
33
34
35
36
37
38
39
40
41

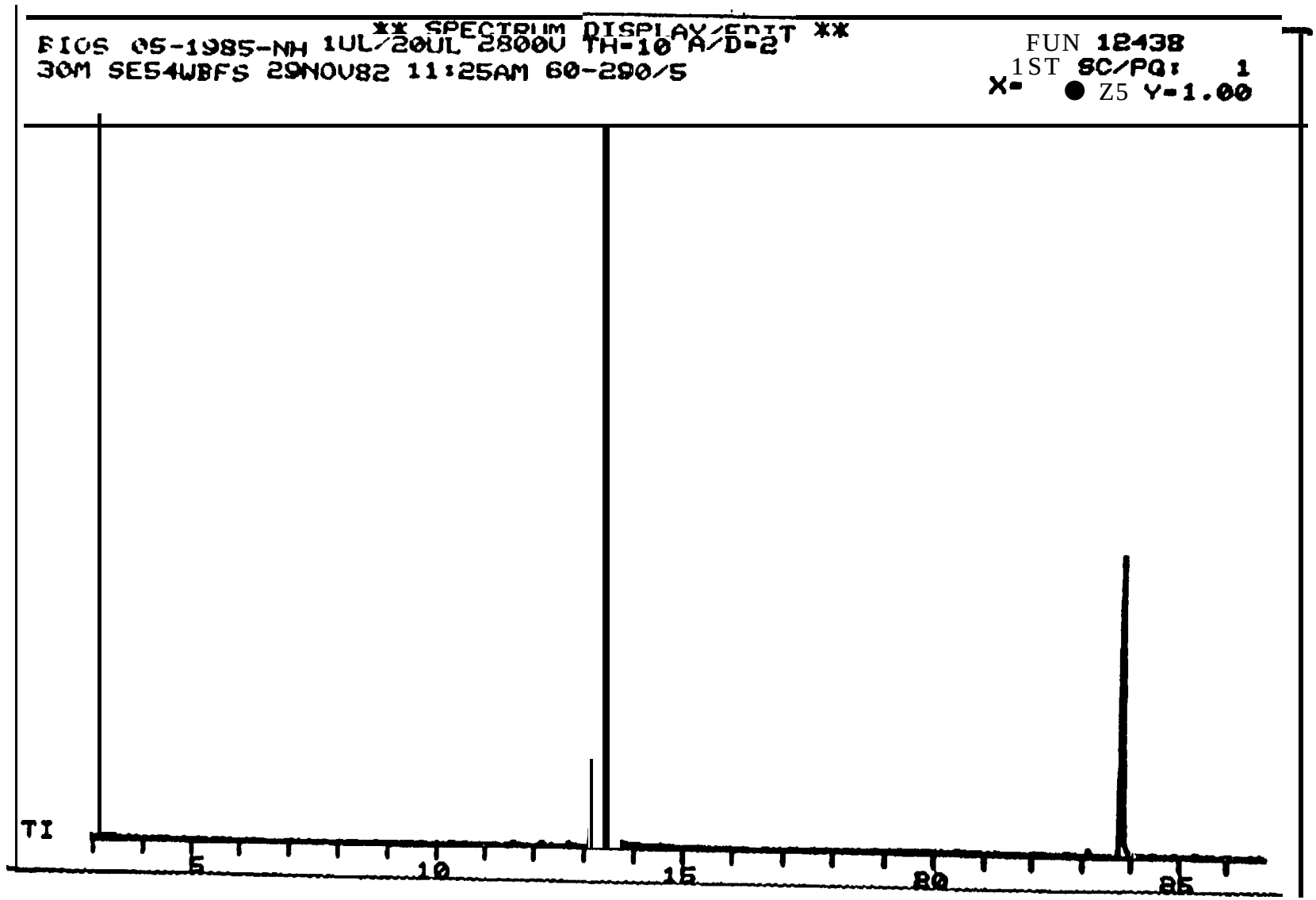
1 23.9 213.8

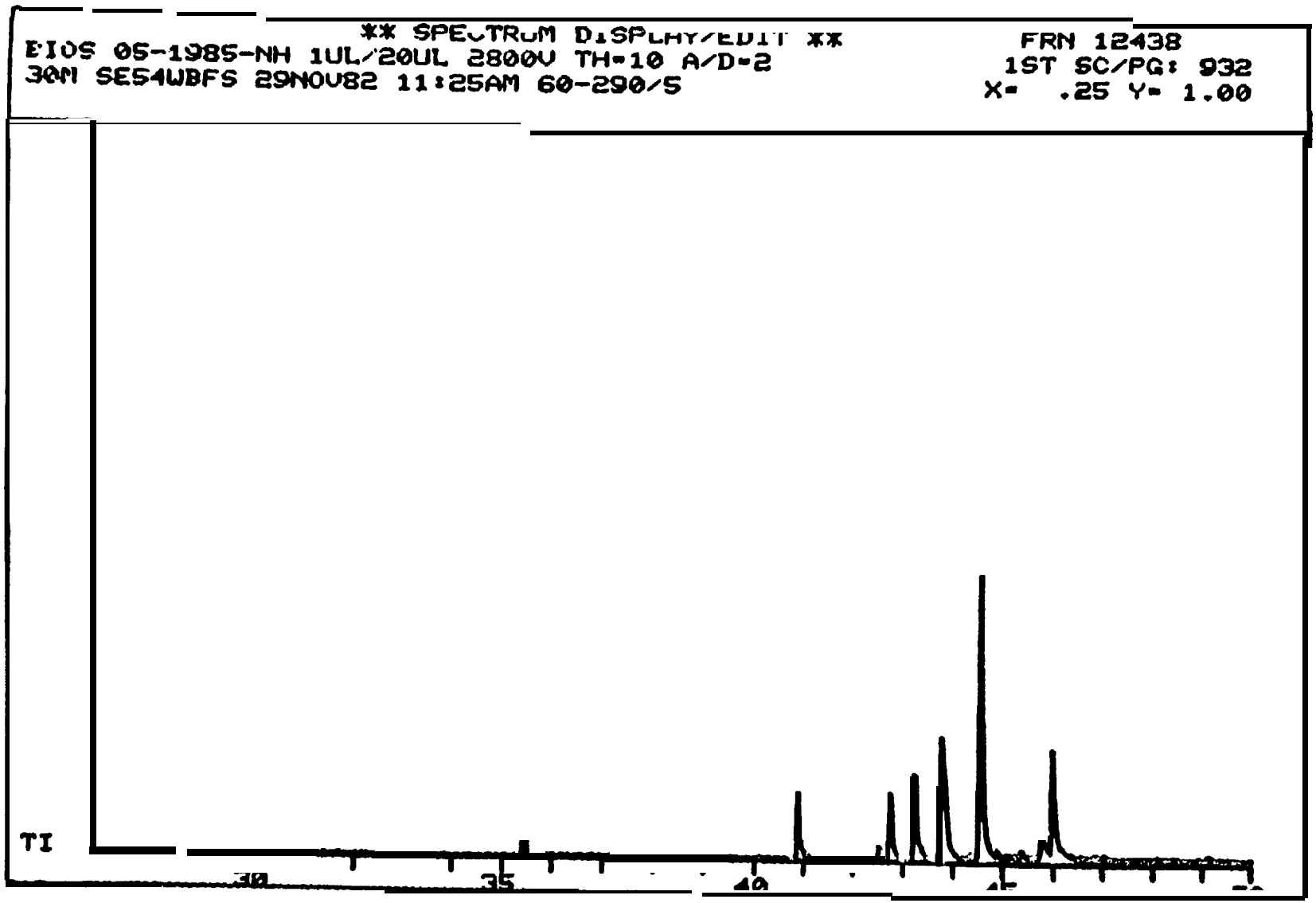
CAL % ON ENTRY?

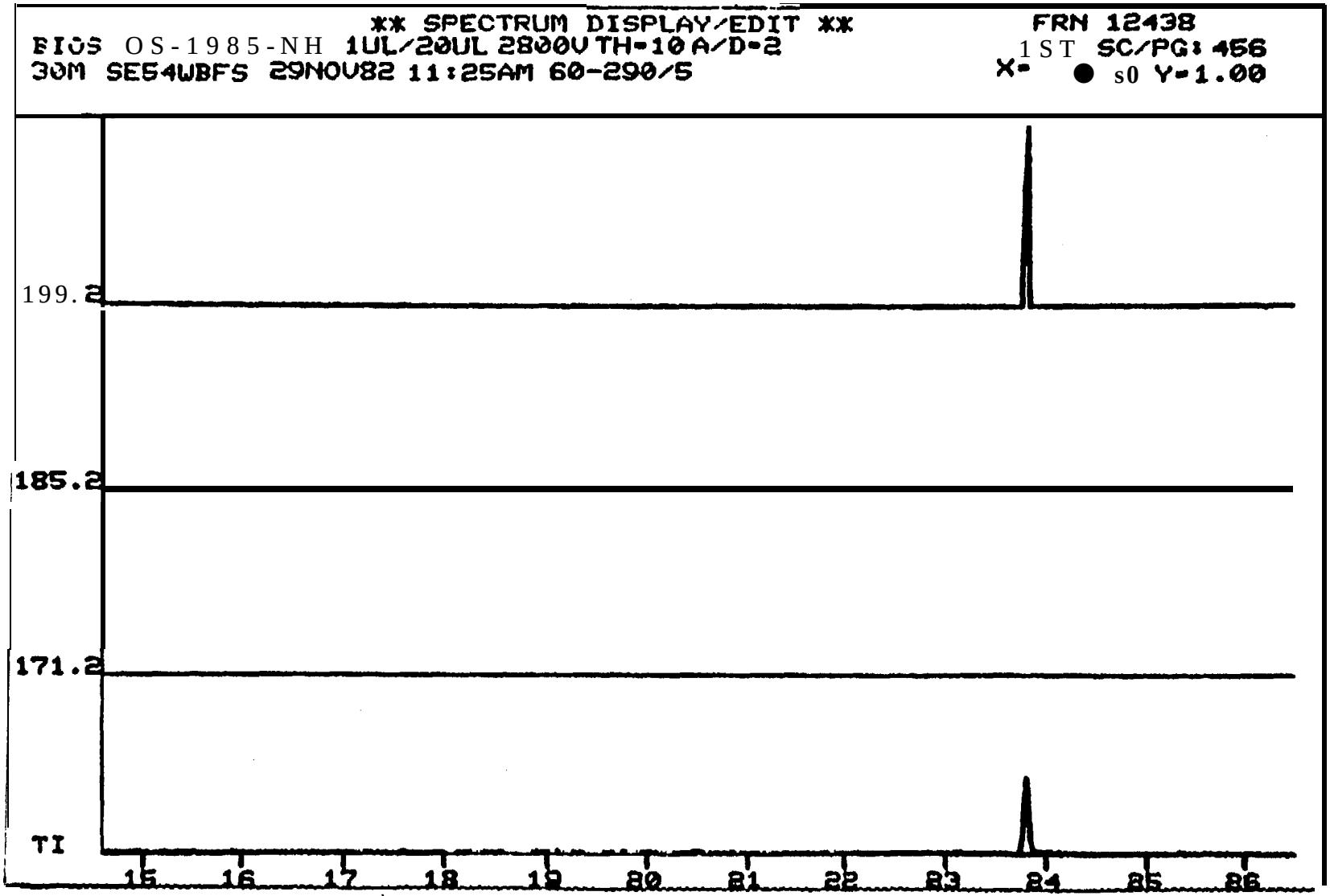


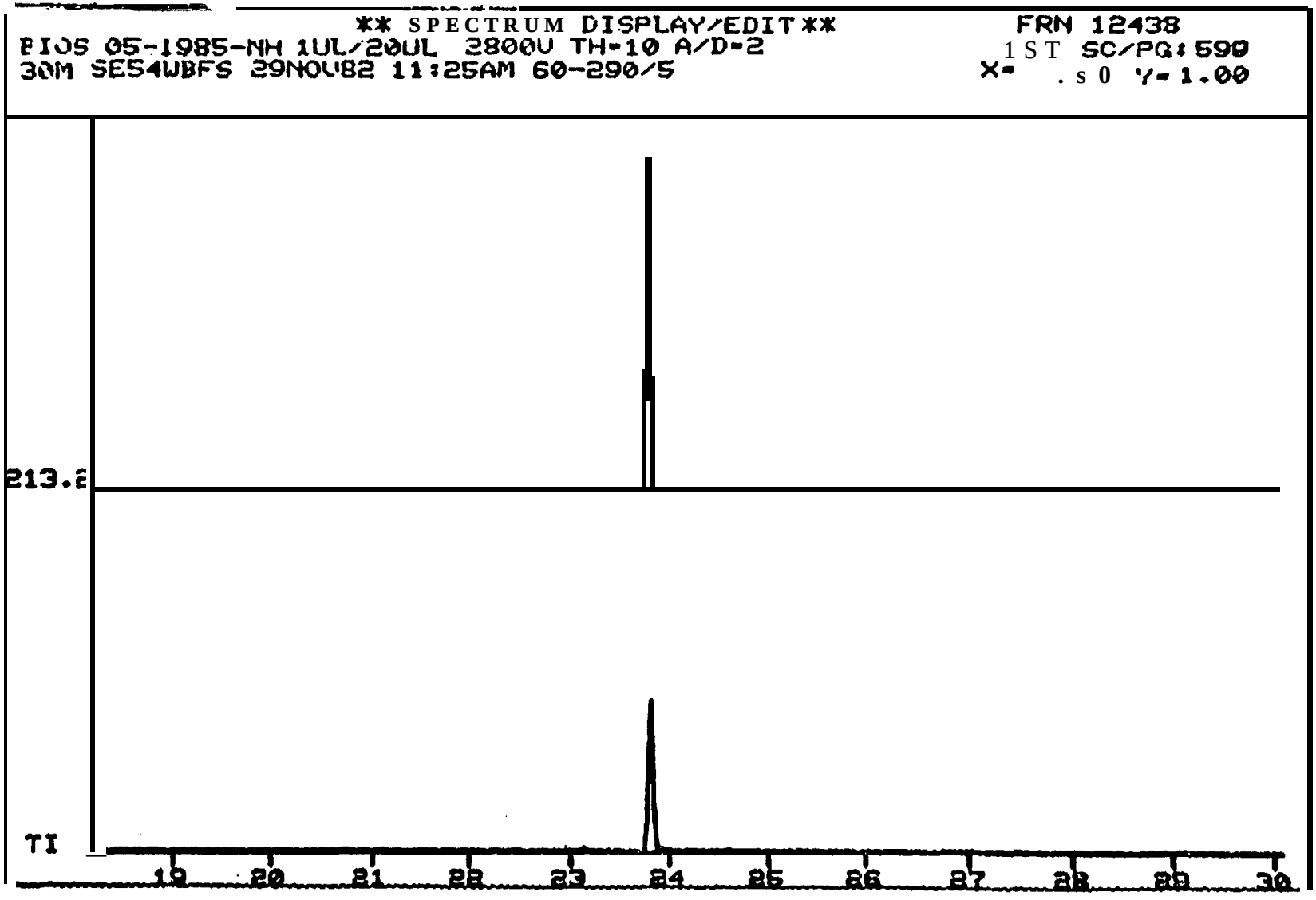
APPENDIX V:

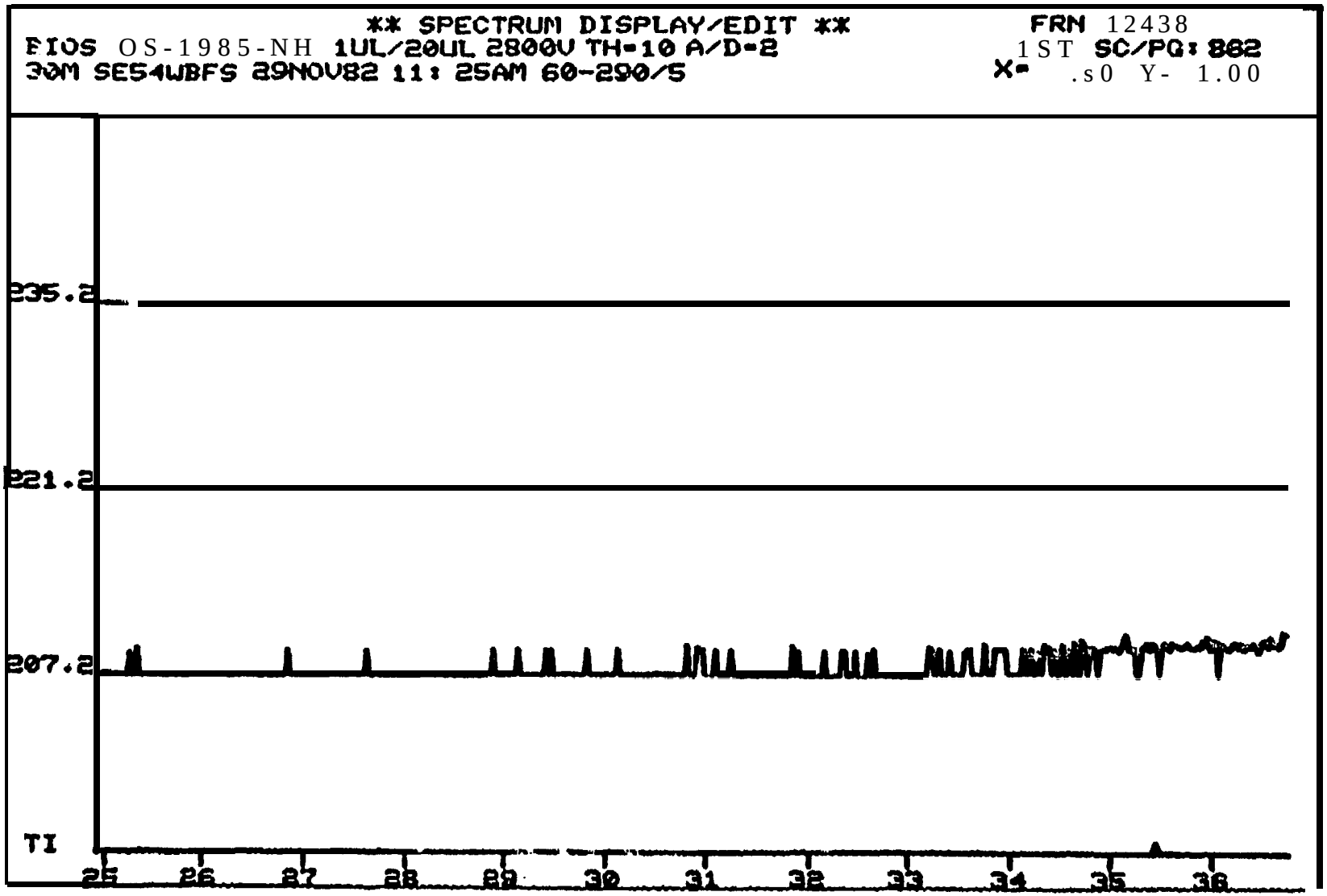
SERRIPES GROENLANDICUS: BAY 10: 2 WEEKS

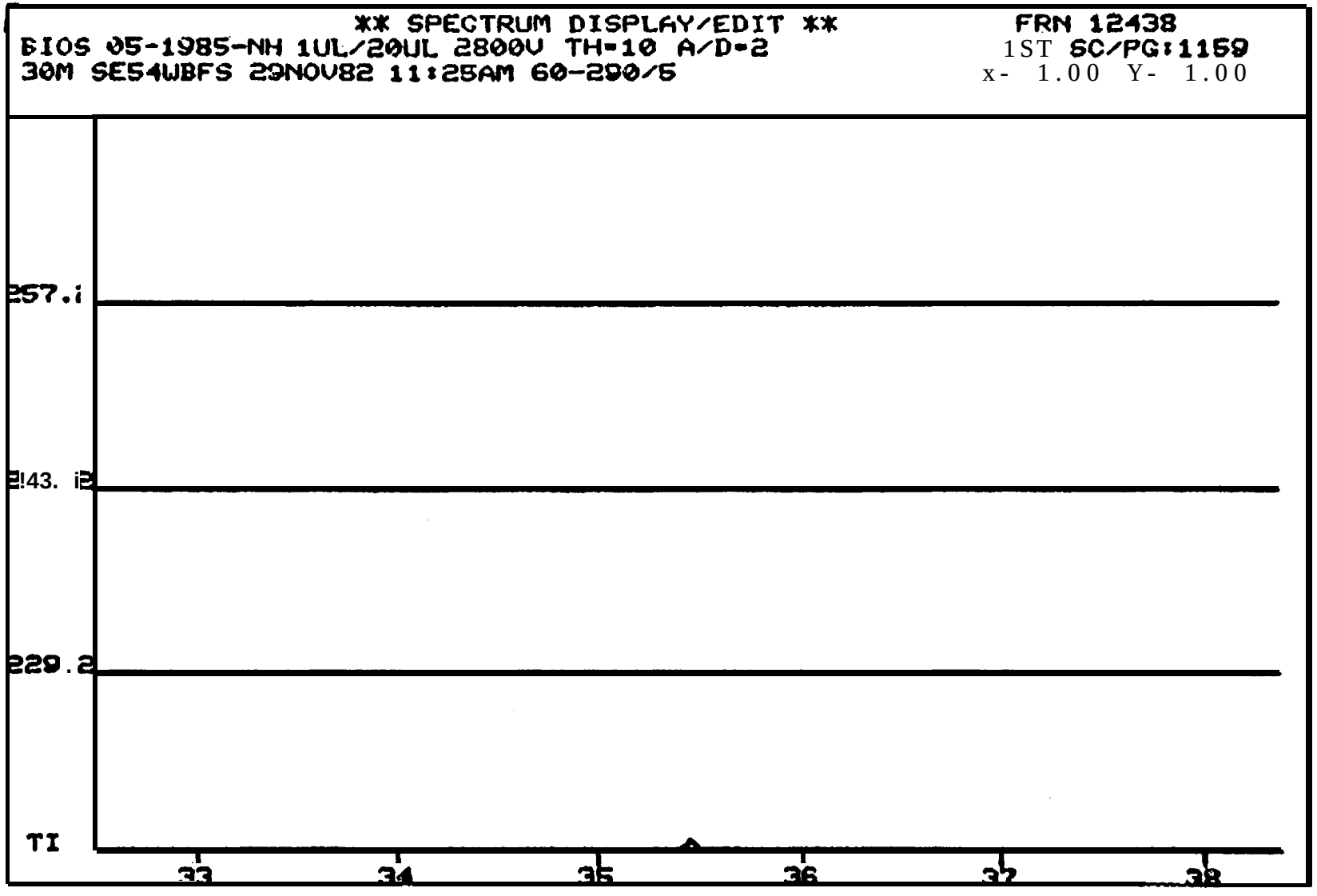

** SPECTRUM DISPLAY/EDIT **
BIOS 05-1985-NH 1UL/20UL 2800V TH-10 A/D-2
30M SES4WBFS 29NOV82 11:25AM 60-290/5
FUN 12438
1ST SC/PQ: 1
X- ● Z5 Y-1.00
TI
5
10
15
80
85

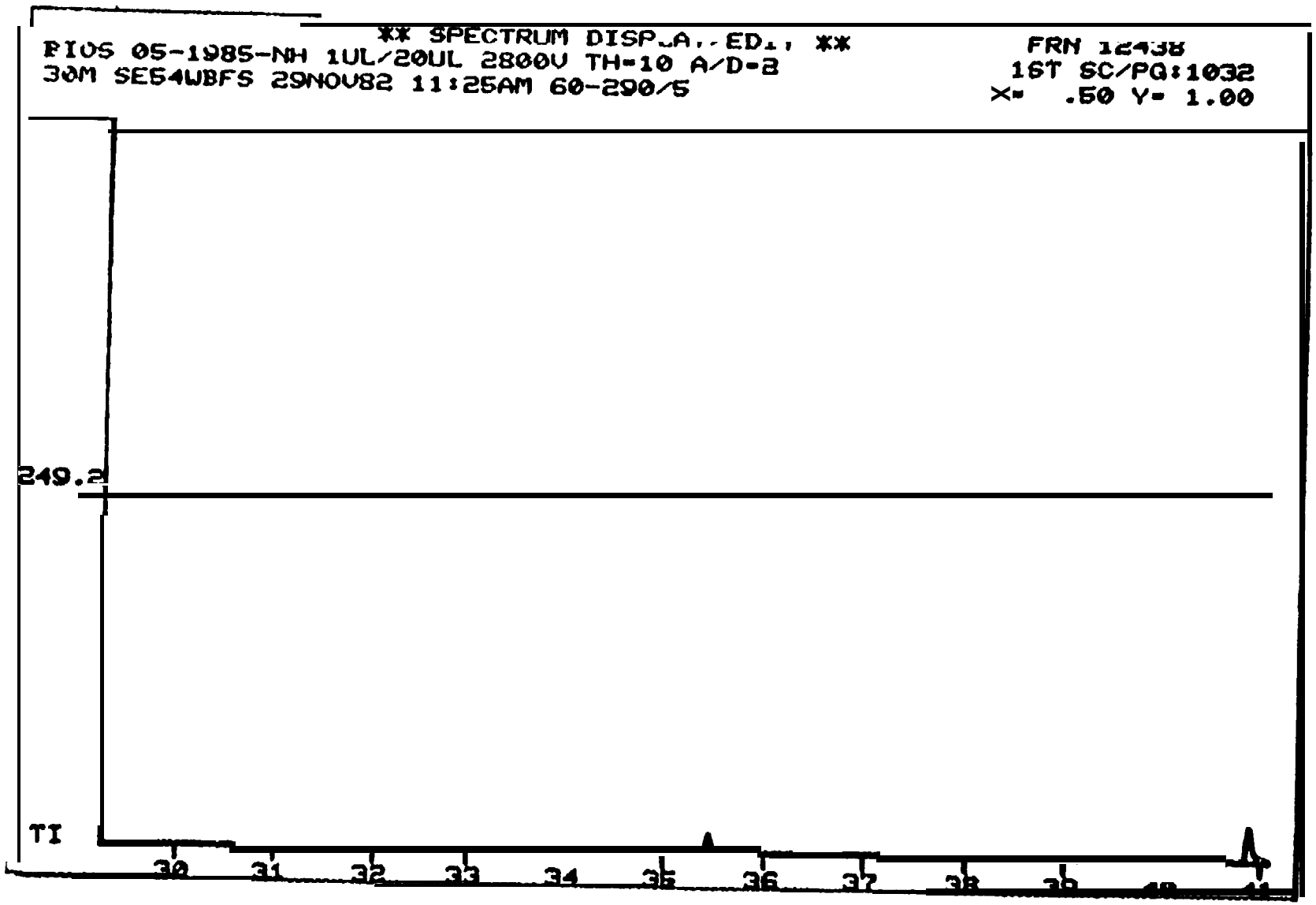

** SPECTRUM DISPLAY/EDIT **
FRN 12438
EIOS 05-1985-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PG: 932
30M SE54WBFS 29NOV82 11:25AM 60-290/5
X= .25 Y= 1.00
TI
30
35
40


** SPECTRUM DISPLAY/EDIT **
FRN 12438
BIOS OS-1985-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PG: 456
30M SE54WBFS 29NOV82 11:25AM 60-290/5
X= ● s0 Y=1.00
199.2
185.2
171.2
TI
15 16 17 18 19 20 21 22 23 24 25 26


** SPECTRUM DISPLAY/EDIT **
FRN 12438
BIOS 05-1985-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PQ: 590
30M SE54WBFS 29NOV82 11:25AM 60-290/5
X= .s0 Y= 1.00
213.2
TI
19
20
21
22
23
24
25
26
27
28
29
30


** SPECTRUM DISPLAY/EDIT **
FRN 12438
FIOS OS-1985-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PQ: 862
30M SE54WBFS 29NOV82 11: 25AM 60-290/5
X= .s0 Y- 1.00
235.2
221.2
207.2
TI
25
26
27
28
29
30
31
32
33
34
35
36


** SPECTRUM DISPLAY/EDIT **
FRN 12438
BIOS 05-1985-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PG:1159
30M SE54WBFS 29NOV82 11:25AM 60-290/5
x- 1.00 Y- 1.00
257.1
243.1
229.2
TI
33
34
35
36
37
38


** SPECTRUM DISPLAYED **
FRN 12438
PIOS 05-1985-NH 1UL/20UL 2800V TH=10 A/D=2
1ST SC/PG:1032
30M SE54WBFS 29NOV82 11:25AM 60-290/5
X= .50 Y= 1.00
249.2
TI
30
31
32
33
34
35
36
37
38
39
40
41